

SIXTH ANNUAL CONFERENCE ON NEW AND RE-EMERGING INFECTIOUS DISEASES

Hosted by

**CENTER FOR ZOOSES RESEARCH
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN**

APRIL 24-25, 2003
Bevier Hall Auditorium and
Holiday Inn Conference Room

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NEW AND RE-EMERGING INFECTIOUS DISEASES

We are very proud to present the Sixth Annual Conference on New and Re-Emerging Infectious Diseases. Our keynote speaker, Dr. Bertram L. Jacobs, of the Department of Microbiology, Arizona State University, will give us an account of the interactions between smallpox and humans, the prospects for improved vaccines, and the evolution of poxviruses. Dr. M. N. Neely will discuss her efforts to identify the interactions that occur between the host and pathogen that lead to activation of virulence mechanism, and contribute to specific streptococcal states, through the use of a zebrafish model of infection. Dr. S. M. Beverley will report advances made in the knowledge of the diverse roles of *Leishmania* surface glycolipids in virulence and survival in the mammalian host. Dr. Ted Hackstadt will show his studies on the interaction of chlamydiae with the secretory pathway of their host cell. Dr. K. Haldar will present her results on the 'sorting' vacuoles of intracellular malaria parasites and *Salmonella*. Dr. K. Kim will talk about subtilisin serine proteases from *Toxoplasma gondii* that have an important role in parasite survival in the host. Dr. S. C. Guptill will show how West Nile virus have spread coast to coast in the U.S. Finally, the Poster Session will give students and faculty at the UIUC and other regional research institutions an opportunity to showcase their research in new and re-emerging infectious diseases. Conference organizers hope that the Conference will stimulate interdisciplinary initiatives to address them and draw much needed attention to the problem of infectious disease.

Acknowledgments

The Conference Committee wishes to thank all that have helped to make this meeting possible. We especially want to acknowledge Dr. Herbert Witheley, Dean, College of Veterinary Medicine, for his support, and Judy Mewes and Debra Domal for their invaluable organizational help. We would like to thank the Veterinary Continuing Education-Public Service (CEPS) office for organizing the space we needed. The financial support of our main sponsors, the Illinois Governor's Venture Technology Fund, the College of Veterinary Medicine, and the Department of Veterinary Pathobiology, greatly contributed to the Conference's success.

Cover: Crow (one of West Nile Virus victims); mosquito (responsible for transmission of many zoonoses); mosquito eggs; ticks (responsible for Lyme disease transmission).

SCHEDULE

Thursday April 24

Bevier Hall Auditorium, 905 S. Goodwin, Urbana, IL 61801

5:00 p.m.: **HERBERT WHITELEY**, Dean, College of Veterinary Medicine, UIUC;
Welcome

5:15-p.m.: **BETRAM L. JACOBS**, Department of Microbiology, Arizona State University,
Tempe, AZ
"The Role of dsRNA and Z-DNA binding proteins in poxvirus
pathogenesis"

6:15-7:15 p.m.: Reception

4:30-6:00 p.m.: Registration (Bevier Hall)

Friday April 25

Holiday Inn, 1001 W. Kilarney, Urbana, IL 61801

7:30-8:15 a.m. Registration/Check-in/Poster Setup
Hall

8:00-8:15 a.m. Conference Introduction and Welcome

Salon D and E

8:15-9:00 a.m. **MELODY N. NEELY**, Department of Immunology and Microbiology, Wayne
State University School of Medicine, Detroit, MI
"Fishing for virulence genes: zebrafish as a model host for
streptococcal pathogenesis "

9:00-9:45 a.m. **STEPHEN M. BEVERLEY**, Departments of Molecular Microbiology,
Washington University School of Medicine, St. Louis, MO

“The diverse roles of *Leishmania* surface glycolipids in survival in the mammalian host”

9:45-10:15 a.m. Coffee break
Hall

10:15-11:00 a.m. **TED HACKSTADT**, Laboratory of Intracellular Parasites, NIAID, National Institutes of Health, Hamilton, MT
“Subversion of host vesicular trafficking pathways by *Chlamydia trachomatis*”

11:00-11:45 p.m. **KASTURI HALDAR**, Departments of Pathology and Microbiology-Immunology
The 'sorting' vacuoles of intracellular malaria parasites and *Salmonella*

11:45-2:30 p.m. Lunch break and poster viewing Hall

2:30-3:15 p.m. **KAMI KIM**, Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY
“Role of subtilisin serine proteinases of *Toxoplasma gondii* in parasite survival”

3:15-4:00 p.m. **STEVE C GUPTILL** U.S. Geological Survey, Reston, VA
“Analyzing the spread of West Nile virus and assessing the risk of human illness”

4:00-6:00 p.m. Reception & Poster Viewing Hall

PRESENTATION ABSTRACTS

THE ROLE OF dsRNA- AND Z-DNA BINDING PROTEINS IN POXVIRUS PATHOGENESIS

Bertram L. Jacobs

Departments of Microbiology, Arizona State University, Tempe, AZ.

Smallpox was one of the most devastating diseases known to humankind, infecting up to 80% of the population during the Middle Ages and killing 20-30% of those infected (it is estimated that in the century before its demise smallpox killed 1 billion human beings). Humans have been trying to protect against smallpox infection for at least 1,000 years, the Chinese having “invented” the idea of giving potential patients a mild case of smallpox which would protect from the naturally acquired disease. Since the turn of the 19th Century we have used several closely related orthopoxviruses (cowpox or vaccine virus) to immunize against smallpox. In fact the vaccine currently available is closely related to the vaccine first used by Jenner in 1796. Despite the relatively high rate of complications associated with this vaccine, smallpox was eradicated from the wild in the 1970s, and now officially only exists in two high security laboratories. It is the likelihood that smallpox also exists unofficially in less secure repositories that has renewed the need for protecting against smallpox. In this talk I will give a history of the interactions between smallpox and humans, will talk about the prospects for improved vaccines and will end with a discussion of evolution of virulence in poxviruses, including our own work on the role of novel nucleic acid binding proteins in poxvirus pathogenesis.

FISHING FOR VIRULENCE GENES: ZEBRAFISH AS A MODEL HOST FOR STREPTOCOCCAL PATHOGENESIS

Melody N. Neely

Immunology & Microbiology Dept., Wayne State School of Medicine, Detroit, MI.

Streptococcal pathogens continue to evade concerted efforts to decipher clear-cut virulence mechanisms, although numerous genes have been implicated in pathogenesis. A single species can infect a diversity of tissues, suggesting the expression of specific virulence factors based on the local tissue environment or stage of infection. The long-range goal of our lab is to identify the interactions that occur between the host and pathogen that lead to activation of virulence mechanisms and contribute to specific streptococcal disease states. To aid us in this pursuit, we have developed a unique animal model, the zebrafish (*Danio rerio*), to characterize specific virulence mechanisms utilized within various tissues *in vivo*. We are using this model host to study infection by two streptococcal species that represent two forms of streptococcal disease: a natural pathogen of fish and humans, *Streptococcus iniae* and a human-specific pathogen, *Streptococcus pyogenes*. *S. iniae* primarily causes a fatal systemic disease in the

zebrafish following intra-muscular injection, with similar pathologies to that seen in human infections caused by *S. agalactiae* and *S. pneumoniae*. While the fatal infection by *S. pyogenes* causes a locally spreading necrotic disease confined to the muscle with pathology similar to what is observed in a human infection of necrotizing fasciitis. By studying pathogens that are virulent for both fish and humans and that mediate disease states in the zebrafish that are identical to those found in human streptococcal infections, we will be able to identify common virulence strategies shared by a number of Gram positive pathogens. Using several genetic strategies with the two streptococcal strains we are currently conducting specific screens in the zebrafish to: (1) identify and characterize cell membrane proteins that interact with the host *in vivo* to cause specific disease states; (2) identify genes required for growth *in vivo*, as well as progressive stages of infection; (3) identify genes that are only expressed while in the host along with tissue specificity of the encoded proteins; and (4) analyze responses of the host that affect progression of disease.

THE DIVERSE ROLES OF *Leishmania* SURFACE GLYCOLIPIDS IN SURVIVAL IN THE MAMMALIAN HOST

Stephen M. Beverley

Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO.

The trypanosomatid protozoan *Leishmania* synthesizes a variety of GPI anchored molecules on its surface. Sorting out their individual functions has been difficult and in some cases controversial as they share structural domains and generally have been tested outside the context of key components such as the major glycolipid Lipophosphoglycan (LPG), phosphoglycans (PGs), ether lipids which constitute approximately 20% of membrane lipids and most GPI anchors, and others. We have studied their role in the context of parasite knockouts and 'add-back' controls, probing their role in diverse aspects of parasitism such as entry, inhibition of phagolysosomal fusion and host signal transduction, and pathogenesis. All of these molecules play critical roles in virulence, although sometimes in unanticipated ways. PGs in particular are required for parasite persistence but not acute pathology, therefore defining a new class of parasite genes important to transmission.

SUBVERSION OF HOST VESICULAR TRAFFICKING BY *Chlamidya trachomatis*

Ted Hackstadt

Laboratory of Intracellular Parasites, NIAID, National Institutes of Health, Hamilton, MT.

Unlike the majority of intracellular parasites which block maturation of endosomes to lysosomes at discrete stages and then replicate within those vacuoles, chlamydiae appear to dissociate themselves from the endocytic pathway shortly after internalization by actively

modifying the vacuole to become fusogenic with sphingomyelin-containing exocytic vesicles. Interaction with a secretory pathway appears to provide a pathogenic mechanism allowing chlamydiae to establish themselves in a site not destined to fuse with lysosomes. Fusion with Golgi-derived vesicles provides a likely source of cellular lipids for the growth of the inclusion membrane as it expands to accommodate the multiplying parasites.

THE 'SORTING' VACUOLES OF INTRACELLULAR MALARIA PARASITES AND *Salmonella*

Kasturi Haldar

Departments of Pathology and Microbiology-Immunology, Northwestern University School of Medicine, Chicago, IL.

Our studies on the malaria parasite focus on protein trafficking, gene expression and drug development. We study vacuolar trafficking of host raft proteins and parasite virulence determinants (by tagging genes with GFP and expressing them by transfection) for their consequence on malarial entry into the red cell, virulence secretion systems and apicoplast biogenesis. The apicoplast is a newly identified residual plastid acquired by secondary endosymbiosis that has attracted attention for its evolutionary novelty and its candidacy as a drug target. Temporal regulation of plasmodial genes may be important for protein targeting in cells. In this context we are examining the role of unique promoter elements and chromatin in regulating expression of secretory determinants such as the histidine-rich proteins and adherence antigens. Finally, whole genome scanning approaches (microarrays and other functional approaches) are being used in combination with informatics to develop novel lipid linked targets for drug development. In our studies on *Salmonella* we are currently examining effectors of the SPI-2 system (*Salmonella* Pathogenicity Island -2 system) for their effects on sterol recruitment, metabolism and bacterial virulence in the mouse model. Since up to 30-40% of total host cholesterol is diverted to the SCV our efforts focus on its source and mechanisms of trafficking to the SCV. We are also investigating the requirement for non-sterol precursors in protecting infected cells from apoptotic and/or necrotic death. Finally microarrays are employed to identify subsets of *S. typhimurium* virulence determinants required for lipid-linked intracellular bacterial replication.

ROLE OF SUBTILISIN SERINE PROTEINASES OF *Toxoplasma gondii* IN PARASITE SURVIVAL

Kami Kim

Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York.

Toxoplasma gondii is a major cause of birth defects and infections in immunocompromised individuals. Like all members of the phylum Apicomplexa, *T. gondii* is an obligate intracellular organism. Micronemes and rhoptries are specialized secretory

organelles of the Apicomplexa whose contents are thought to be essential for successful invasion of host cells. We have identified two subtilisin-like serine proteinases from *T. gondii*, TgSUB1 and TgSUB2, that we hypothesize are necessary for successful invasion. Serine proteinase inhibitors have been reported to block host cell invasion by both *T. gondii* and the related apicomplexan parasite *P. falciparum*. Disruption of *TgSUB2* was unsuccessful implying that *TgSUB2* is an essential gene. Both TgSUB1 and TgSUB2 undergo autocatalytic processing as they traffic through the secretory pathway. TgSUB1 is a microneme protein whereas TgSUB2 localizes to rhoptries and associates with rhoptry protein ROP1, a potential substrate. Mutational analysis suggests that TgSUB2 is a rhoptry protein maturase. Processing of secretory organelle contents appears to be ubiquitous among the Apicomplexa. As subtilases are present in genomes of all the Apicomplexa sequenced to date, subtilases may represent a novel chemotherapeutic target.

ANALYZING THE SPREAD OF WEST NILE VIRUS AND ASSESSING THE RISK OF HUMAN ILLNESS

Stephen C. Guptill, Susan D. Price, Lesley E. Milheim, Michelle F. Coffey, and F. Lee De Cola

U.S. Geological Survey, Reston, VA.

Since it was first detected in New York City in 1999, West Nile (WN) virus has spread coast-to-coast, having been found in 43 States from Maine to California. The U.S. Geological Survey is working with the Centers for Disease Control and Prevention (CDC) to learn the current geographic extent of the WN virus, to understand how it moves between birds, mosquitoes, and humans, and to predict future outbreaks of the virus. A collaborative 3-year research project is being conducted on U.S. Fish and Wildlife Service, National Park Service, other Federal lands, and on State, local, and private lands along the Atlantic and Mississippi Flyways. This study uses the sampling of migratory and local wild birds to detect the presence of WN virus and identify possible avian carriers. Over 10,000 birds of more than 150 species have been captured, sampled, and released at 20 Federal and 3 other sites in 12 States during the spring and fall bird migration seasons of 2001 and 2002. A parallel study, being conducted with CDC, is examining the distribution and number of mosquito species in relation to land cover, weather conditions, and avian mortality. Systematic mosquito surveillance (weekly collections at seven sites) is being conducted year-round in St. Tammany Parish in Louisiana, complementing avian collections done at Bogue Chitto and Big Branch National Wildlife Refuges in the parish. Finally, West Nile virus surveillance data from the CDC is being studied to determine the spatial and temporal relationships between disease outbreaks in birds and animals and human illness. Information from these analyses will guide the creation of predictive models of disease risk. These surveillance systems provide the basic information on the “geography” of the virus. Combining these data with information about avian migratory patterns, landscape characteristics, and weather conditions, over space and time, will provide the foundation for developing spatial analytical and forecasting models to assess the risk of human illness.

POSTER ABSTRACTS

1 MODIFIED VIRUS ANKARA ACTIVATES NUCLEAR FACTOR-KAPPA B THROUGH THE MEK/ERK PATHWAY

Roderick J. Gedey and Joanna Shisler

Department of Microbiology, College of Medicine, University of Illinois at Urbana-Champaign.

Poxviruses are large DNA viruses that replicate in the cytoplasm of host cells. Recent events concerning the use of smallpox as a bioterrorist weapon and renewed smallpox vaccinations have emphasized the need for a safer smallpox vaccine. At the end of the global smallpox vaccination campaign led by WHO, Modified Virus Ankara (MVA) was used successfully to vaccinate 100,000 people with no reported complications. MVA is a strain of vaccinia virus whose parental strain Ankara was passaged in chick embryo fibroblasts (CEFs) over 500 times, resulting in the deletion of many of the parental genes. These genes include host range genes, limiting the host range of MVA to permissively infect to CEF and BHK-21 cell lines. Other deleted genes include classical poxviral immune evasion genes. It has been shown that the parental vaccinia virus strain can inhibit the activation of the host immune regulatory transcription factor nuclear factor-kappaB (NF- κ B) by interfering with the degradation of the inhibitor of NF- κ B (I κ B). In contrast, the less virulent MVA activates NF- κ B. In this study we show that MVA induces I κ B degradation across many cell lines regardless of cell permissibility, including HeLa, HEK 293T, BHK-21, RK13, and Jurkat. To determine the viral gene responsible for inducing NF- κ B activation, we infected cells with MVA in the presence of the nucleoside analog cytosine arabinoside (araC), an inhibitor of intermediate and late gene expression, or infected cells with MVA pretreated with psoralen/UV to inhibit early, intermediate, and late gene expression. These results show that an early gene product activates NF- κ B. To define the signal transduction pathway MVA activates we infected HEK 293T cells in the presence of the pharmacological agents PD98059, U0126 (inhibitors of MEK), SB203580 (p38 MAPK inhibitor), LY294002, wortmannin (inhibitors of PI3K), AG1478 (inhibitor of EGFR signaling) and rapamycin (inhibitor of p70S6K). We also used a dominant negative form of I κ K β . Treatment with PD98059, U0126, or I κ K β -/- inhibited MVA induced NF- κ B activation while the other treatments did not. From these data we hypothesize that the viral gene product responsible for NF- κ B activation acts solely on the MEK/ERK pathway. Further studies using genetic, computational, and functional analysis will elucidate the viral protein responsible for the activation of NF- κ B.

2 ARE LIFE HISTORY CHARACTERISTICS ASSOCIATED WITH PREVALENCE OF WEST NILE VIRUS INFECTION IN BATS?

Jennifer Miriam Wise, Nohra E. Mateus-Pinilla, Robert Novak, Joyce E. Hofmann, Edward J. Heske

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West Nile Virus (WNV) has caused 62 human fatalities in Illinois alone and significant morbidity and mortality in mammals and birds in the US. WNV has been isolated from insectivorous bats (big brown bat, *Eptesicus fuscus*, and little brown bat, *Myotis lucifugus*) from New York. Bats infected by St. Louis encephalitis, a virus related to WNV, has been shown to be capable of maintaining active viremia while in hibernacula. The aim of this study is to determine if bats have been infected with WNV in Illinois, and if their life history characteristics are associated with prevalence of infection. The long-term goal is to determine whether bats have the potential to play a role in the life cycle of West Nile Virus. Red bats, (*Lasiurus borealis*), and big brown bats are studied due to their similar size and body weight, but different natural history characteristics (i.e. red bats are solitary, migratory and confined mainly to woody areas while big brown bats are gregarious, hibernating residents of Illinois and regularly visit urban areas and barns). Bats were captured throughout Illinois between May 29th, and November 4th, 2002. Field work was done on 19 occasions yielding a total of 22 bats captured and 14 serum samples. Bats were captured at night using mist nets and during the day from roost sites and maternity colonies. A preliminary hibernacula study was performed in 6 mines and caves yielding a total of 13 blood samples from big brown bats (red bats are presumed not found in Illinois at this time of year). Serum samples were tested for WNV antibody using blocking ELISA. In addition, approximately 75 bats collected by the Illinois Department of Public Health between January 2001 and September 2002 have been necropsied. Kidney, liver, and heart samples were collected and tested using TaqMan Reverse Transcriptase-PCR assay for evidence of WNV. All serum and tissue samples have been negative for WNV infection. The sample size for the study remains small and at this time we can not draw definitive conclusions from our results. However, we are preparing for a second year of field and lab work in 2003

3 STRAIN VARIATION IN AN EMERGING IRIDOVIRUS

Tony L. Goldberg ^{1,3*}, David A. Coleman ¹, Emily C. Grant ², Kate R. Inendino ², David P. Philipp ³

¹ Department of Veterinary Pathobiology, and ² Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign; ³ Illinois Natural History Survey, Center for Aquatic Ecology, Champaign. *Corresponding author.

Although iridoviruses vary widely within and among genera with respect to their host range and virulence, variation within iridoviral species has been less extensively characterized. This study explores the nature and extent of intraspecific variation within an emerging iridovirus of North American warmwater fishes, largemouth bass virus (LMBV). Three LMBV isolates recovered from three distinct sources differed genetically, and phenotypically *in vitro* and *in vivo*. Genetically, the isolates differed in the banding patterns

generated from amplified fragment length polymorphism analysis, but not in their DNA sequences at two loci of different degrees of evolutionary variability. *In vitro*, the isolates varied in the rate at which they induced cytopathic effect in fathead minnow cells, but not in the rate at which they replicated, as determined by real-time quantitative PCR. *In vivo*, the isolates varied over five-fold in virulence, as measured by the rate at which they induced mortality in juvenile largemouth bass. This variation was reflected in the viral load of exposed fish, measured using real-time quantitative PCR; the most virulent viral strain also replicated to the highest level in fish. Together, these results justify the designation of these isolates as different strains of LMBV. Strain variation in iridoviruses could help explain why animal populations naturally infected with iridoviral pathogens vary so extensively in their clinical responses to infection. The results of this study are especially relevant to emerging iridoviruses of aquaculture systems and wildlife.

4 ACID TOLERANCE OF *Shigella boydii* 18: PHYSIOLOGICAL AND MOLECULAR ANALYSIS OF *S. boydii* FOODBORNE OUTBREAK STRAIN COMPARED TO RELATED ENTERIC BACTERIA

Yvonne C. Chan and Hans P. Blaschek

Food Microbiology Division, Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign.

Shigella boydii is a food pathogen that was implicated in a 1998 foodborne outbreak in bean salad. Imported parsley and cilantro from Mexico were the suspected food vehicles since *S. boydii* is not a pathogen commonly found in the United States. Previous studies have shown that *S. boydii* can survive in bean salad which contains organic acids and has a decreasing pH over time (Agle and Blaschek, unpublished data). Acid challenge assays in acidified tryptic soy broth (TSB) (pH 4.5) and acidified M9 minimal salts media (pH 2.5) with amino acids, arginine or glutamic acid, have been performed on *S. boydii*, *Shigella flexneri*, and *Escherichia coli* 0157:H7 strains to compare differences in their acid resistance systems. Differences in acid survival of logarithmic phase cells were detected in acidified LB at pH 4.5. In acidified minimal media containing arginine, *S. boydii* strains were able to survive at pH 2.5. The arginine decarboxylase (*adi*) gene is present in *S. boydii* and is involved in survival at extremely low pH. The discovery of the *adi* gene in *S. boydii* is significant because the arginine decarboxylase gene was thought to be unique to *E. coli* (Foster and Moreno, 1999). Also, sequencing of the *rpoS* gene from the *S. boydii* outbreak strain indicates that it is 99% conserved when compared to the *E. coli* K-12 *rpoS* gene and plays a vital role in acid survival.

5 MICROSCOPIC EXAMINATION OF *Shigella boydii* 18 ON PARSLEY AND CILANTRO

M.E. Agle¹, S. Ragengpradub¹, C. Conway², S.J. Robinson² and H.P. Blaschek¹

¹Department of Food Science and Human Nutrition, and ²Imaging Technology Group, Beckman Institute, University of Illinois Urbana-Champaign.

Produce-related outbreaks have increased drastically over the past twenty years. *Shigella boydii* 18 was isolated from a 1999 Chicago area foodborne outbreak involving contaminated bean salad that contained fresh parsley and cilantro. Cilantro and parsley were also implicated as the food vehicles in a 1998 multi-state outbreak of *Shigella sonnei*. We have previously shown that *S. boydii* 18 is capable of surviving on parsley treated with commercial produce wash or with water. When parsley was inoculated with 10^6 CFU/g *S. boydii* 18, water or produce wash yielded 1.38 and 1.36 log reductions, respectively. Alternatively, when produce wash was inoculated with *S. boydii* no organisms were detected after one or five minutes. The objective of this work is to explain the reduced efficacy of produce wash on *Shigella* on produce. Scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM) were used to observe *S.boydii* 18 on parsley and cilantro samples treated with produce wash and water. Parsley and cilantro samples were inoculated by dipping in a *Shigella* horse serum suspension (optical density = 1.5). Samples were allowed to dry in a laminar flow hood for 1.5hrs and then were treated with water or produce wash for five minutes. Samples were fixed with 2% glutaraldehyde, and stained with 2% osmium tetroxide, dehydrated using a graded ethanol series and critical point dried using hexamethyldisilazane. Samples were sputter coated with gold/palladium and imaged using a Philips XL30 ESEM-FEG microscope. Evidence of biofilm formation by *S. boydii* was observed on the parsley and cilantro samples. Cells in biofilms have been found to be more resistant to antibiotics and disinfectants. This may explain the decreased efficacy of produce wash on parsley and cilantro samples.

6 IDENTIFICATION OF ACIDOCALCISOMES IN *Rhodospirillum rubrum*

Manfredo Seufferheld, Mauricio Vieira, and Roberto Docampo
Laboratory of Molecular Parasitology, Department of Veterinary Pathobiology,
University of Illinois at Urbana-Champaign.

Volutin granules or polyphosphate bodies are widespread in organisms from different phylogenetic groups. These structures have been described as electron dense granules containing polyphosphate. In recent years volutin granules of unicellular eukaryotes, such as *Chlamydomonas reinhardtii*, *Dictyostelium discoideum*, and a number of human pathogens including malaria parasites, *Toxoplasma gondii*, and trypanosomatids, were found to possess an enclosing membrane with a number of pumps and exchangers and were named acidocalcisomes. Moreover, we found recently that the volutin granules of *Agrobacterium tumefaciens* are homologous of the acidocalcisomes of early eukaryotes (Seufferheld et al., 2003, submitted). In this work we demonstrate that the “bacterial acidocalcisomes” are present in other species of bacteria, such as the photosynthetic bacterium *Rhodospirillum rubrum*. The volutin granules of *R. rubrum* are similar to the acidocalcisomes found in *A. tumefaciens* and early eukaryotes, as indicated by: (1) electron microscopy of intact bacteria and the “granule fraction”; (2) X-ray microanalysis of the volutin granules that revealed the presence of large amounts of phosphorus, calcium,

magnesium, and potassium; (3) the increase in the calcium content of the “granules” when bacteria were cultured in Ca^{2+} rich medium; (4) immunofluorescence microscopy with 3-D reconstruction, and immunoelectron microscopy using antibodies against a vacuolar H^+ -PPase present in *R. rubrum* and similar to the acidocalcisomal enzyme of early eukaryotes, that revealed labeling of these “granules” inside the bacteria; (5) subcellular fractionation of *R. rubrum* using a modification of a method developed for the isolation of acidocalcisomes of early eukaryotes, which revealed the presence of a dense fraction containing an AMDP-sensitive H^+ -PPase and large amounts of polyphosphate. Our findings support the idea that complex cellular structures appeared before the divergence of prokaryotes and eukaryotes. The elucidation of the function of bacterial acidocalcisomes may have important and widespread implications on important issues such bacterial pathogenesis, their adaptive mechanisms to changing environments and the evolution of prokaryotic and early branching eukaryotic organisms.

7 STRUCTURE AND FUNCTION OF BACTERIAL DERMONECROTIC TOXINS

Melinda J. Faulkner, Timothy J. Bauler, Mengfei Ho, and Brenda A. Wilson
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Pasteurella multocida toxin (PMT), the *E. coli* cytotoxic necrotizing factors 1 and 2 (CNF1, CNF2), and *Bordetella* dermonecrotic toxin (DNT) belong to a family of dermonecrotic toxins that exhibit their effects on cells through the activation of G proteins. DNT and the CNFs constitutively activate the small G protein RhoA by deamidation or transglutamination of Gln63. PMT initially activates $\text{Gq}\alpha$ and subsequently uncouples it from its receptor partner. DNT shares 50% similarity over a 130 amino acid residue region in its C-terminus with the CNFs. PMT and DNT share 18% similarity in their N-termini over a 260 amino acid residue region. PMT and the CNFs share 25% similarity in their N-terminal 500 amino acids. Various approaches were used to determine the structure and function of PMT, DNT, and the CNFs. Deletion mutants and fusion proteins with GFP were constructed for PMT and DNT. Fluorescence microscopy studies with full-length DNT and GFP-labeled DNT were performed to examine the cytotoxicity and transport routes taken by DNT during intoxication. The nature of the cellular receptors for DNT, CNF, and PMT was also studied by using binding assays of [^{125}I]-labeled toxins to purified gangliosides and cellular membrane lipid extracts separated by thin layer chromatography. A procedure for isolating $\text{Gq}\alpha$ for mass spectral analysis was developed for further analysis of the catalytic activity of PMT. Together these studies provide insight into the mechanisms of bacterial toxin activity..

8 EVIDENCE OF TRANSMISSION OF *Brucella abortus* FROM CATTLE TO INDIGENOUS FARM DOGS IN KOREA

B.K. Baek, C.W. Lim, M.S. Rahman, C.-H. Kim, A. Oluoch and I. Kakoma
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Three dogs reared on a dairy farm with a high incidence for *Brucella abortus* were serologically positive for *B. abortus* and no other *Brucella* spp. The identity of the organism was confirmed to be *B. abortus* by AMOS-PCR with specific primers for *B. canis*. 100% homology of the canine isolate and the bovine pathogen isolated from the farm was demonstrated. The only possible source of infection was infected cattle on the same farm. It is suggested that dogs be routinely included in brucellosis surveillance and eradication programs.

9 SUCCESSFUL TRANSPLACENTAL TRANSMISSION OF *Brucella abortus* INFECTION IN SPRAGUE-DAWLEY RATS USING PCR

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The vertical transmission of *Brucella abortus* in Sprague-Dawley (SD) rats was verified using PCR, serology and bacteriological methods concurrently. *Brucella* free SD rats weighing 200-250 grams were subcutaneously injected with 500 µl containing 1 x 10⁹ colony forming units (cfu) suspension of *B. abortus* biotype 1 Korean isolate were allowed to mate with uninfected female partners. *B. abortus* biotype 1 was detected both bacteriologically and by AMOS PCR (*abortus*, *melitensis*, *ovis*, *suis*) technique from testis of infected male rats and spleens of infected female rats. By 7 days post inoculation both the Rose Bengal test (RBT) and the Plate Agglutination test (PAT) were positive. The reciprocal antibody titre of the one-month old litter mates reached 1:400 by RBT and 1:200 by PAT, and 1:800 in the mother rats both by RBT and PAT. The infected female and male SD rats successfully transmitted *Brucella* organisms to their opposite sex partner and to their offspring. Fetuses born to infected dams were found to be infected at 20 days and the AMOS-PCR was shown to be a valuable tool for verification of the vertical transmission of *B. abortus* type 1. These data are discussed in the context of domestic animal-wild animal brucellosis transmission cycle.

10 A GPI MANNOSYLTRANSFERASE THAT REPRESENTS A POTENTIAL DRUG TARGET IN THE HUMAN PATHOGENIC FUNGUS *Candida albicans*

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Biosynthesis and attachment of GPIs to protein is essential in *Saccharomyces cerevisiae*. Although the pathway for assembly of the core of the GPI precursor is conserved among eukaryotes, the yeast pathway also includes the addition of a side branching fourth, α 1,2-linked Man to the Man3-containing core glycan. We previously showed that the essential Smp3 protein is responsible for the addition of the fourth Man in *S. cerevisiae* and that Smp3p acts on Man3-GPIs before transfer to the third Man of the phosphoethanolamine (EthN-P) through which the GPI is linked to protein. In mammals, a fourth mannosylation step appears dispensable because Man3-GPI precursors receive EthN-P on their third mannose. The Smp3p-dependent mannosylation step in fungal GPI assembly is therefore a potential target for agents against human pathogenic fungi. We have cloned an SMP3-like gene from the human opportunistic pathogen *Candida albicans* and have shown in two ways that its product is functionally equivalent to *S. cerevisiae* Smp3p. First, CaSMP3 restores viability to *S. cerevisiae* *smp3 null* mutants and second, expression of CaSMP3 in the *S. cerevisiae* *smp3/gpi13* double mutant leads to *in vivo* conversion of Man3-GPI precursors to Man4-GPIs. We have attempted to disrupt both copies of SMP3 in *C. albicans*, a diploid organism with no sexual cycle, but have been unable to isolate homozygous *Casmp3* disruptants, suggesting that CaSMP3 is essential. To determine whether depletion of CaSmp3p leads to a block in assembly of *C. albicans* GPIs, we have constructed a heterozygous *Casmp3* disruptant in which expression of the remaining copy of CaSMP3 is controlled by the glucose-repressible MAL2 promoter. Upon shift to glucose, this strain accumulates a presumed GPI biosynthetic precursor with a chromatographic mobility similar to that of the Man3-GPI that accumulates in *S. cerevisiae* *smp3* mutants. Structural characterization of this lipid is underway. Our ability to create *C. albicans* strains with conditional defects in GPI assembly will permit analyses of the biochemical and phenotypic consequences of a blocking GPI assembly in this human pathogenic fungus. (Supported by a Burroughs Wellcome Scholar Award in Molecular Pathogenic Mycology to P.O.)

11 FORMATION AND REMODELING OF INOSITOLPHOSPHOCERAMIDE DURING THE DIFFERENTIATION OF TRYPOMASTIGOTE INTO AMASTIGOTE FORMS OF *Trypanosoma cruzi*

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Differentiation of trypomastigote into amastigote forms of *Trypanosoma cruzi* inside myoblasts, or *in vitro*, at low extracellular pH, in the presence of [³H]-palmitic acid or [³H]-inositol, revealed differential labeling of inositolphosphoceramide and phosphatidylinositol,

suggesting that a remodeling process takes place in both lipids. Using [³H]-labeled inositolphosphoceramide and phosphatidylinositol as substrates we demonstrated the association of at least five enzymatic activities with the membranes of amastigotes and trypomastigotes. These included: phospholipase A₁, phospholipase A₂, inositolphosphoceramide-fatty acid hydrolase, acyltransferase and a phospholipase C releasing either ceramide or a glycerolipid from the inositolphospholipids. These enzymes may be acting in remodeling reactions leading to the anchor of mature glycoproteins or glycosylinositolphospholipids, and helping in the transformation of the plasma membrane, a necessary step in the differentiation of slender trypomastigotes to round amastigotes. Synthesis of inositolphosphoceramide and particularly of glycosylinositolphospholipids was inhibited by aureobasidin A, a known inhibitor of fungal inositolphosphoceramide synthases. The antibiotic impaired the differentiation of trypomastigotes at acidic pH, as indicated by increased appearance of intermediate forms and decreased expression of the Ssp-4 glycoprotein, a characteristic marker of amastigote forms. Aurebasidin A was also toxic to differentiating trypomastigotes at acidic pH but not to trypomastigotes maintained at neutral pH. Our data suggest that inositolphosphoceramide is implicated in *T. cruzi* differentiation and its metabolism could provide important targets for the development of antiparasitic therapies.

12 *Trypanosoma cruzi* PHOSPHOINOSITIDE-SPECIFIC PHOSPHOLIPASE C (TcPI-PLC) IS INVOLVED IN DIFFERENTIATION OF TRYPOMASTIGOTES INTO AMASTIGOTES AND IS TARGETED TO THE PLASMA MEMBRANE BY ITS LIPID MODIFIED N-TERMINAL SEQUENCE

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The phosphoinositide-specific phospholipase C (PI-PLC) enzyme is an important component of the inositol phosphate/diacylglycerol signaling pathway. A newly discovered *Trypanosoma cruzi* PI-PLC (TcPI-PLC) is believed to play a role in cell cycle differentiation of the parasite. *TcPI-PLC* is expressed in epimastigotes where it is localized to the cytosol and during the differentiation of trypomastigotes to amastigotes where it localizes to the plasma membrane of amastigotes. The first 20 amino acids of the *N*-terminus of TcPI-PLC contain a dual *N*-myristoylation and palmitoylation consensus sequence that may play a role in targeting and attachment to the plasma membrane. To investigate the importance of the *N*-terminus in targeting, gene fusions were constructed using the *T. cruzi* expression vector pTEX which consisted of the coding sequences for the first 6, 10, or 20 amino acids of the *N*-terminus of TcPI-PLC followed by the entire green fluorescent protein (GFP) sequence. Expression of fusion proteins was confirmed by Western blotting. Fluorescence microscopy confirmed that all 20 amino acids in the *N*-terminal sequence of TcPI-PLC are needed for targeting to the plasma membrane. To determine if *TcPI-PLC* is involved in the differentiation of trypomastigotes to amastigotes, antisense inhibition was performed using phosphorothioate-modified oligonucleotides. Trypomastigotes were preincubated in

medium containing 20 μ M antisense oligonucleotides and then induced to differentiate into amastigotes by exposure to pH 5.0. Antisense oligonucleotide-treated parasites showed a reduced rate of differentiation in comparison to controls, as well as accumulation of intermediate forms, suggesting that TcPI-PLC is involved in this process.

13 CLONING AND CHARACTERIZATION OF *Trypanosoma brucei* PHOSPHATIDYLINOSITOL-PHOSPHOLIPASE C

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Phosphoinositide-specific phospholipase C (PI-PLC) plays critical roles in the eukaryotic signal transduction cascades, since this enzyme catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) to inositol-1,4,5-trisphosphate (IP₃) and 1,2-diacylglycerol (DAG). Here, we report the cloning and characterization of PI-PLC from *Trypanosoma brucei*, the etiologic agent of African sleeping sickness. The isolated cDNA clone of *T. brucei* PI-PLC (*TbPI-PLC*) encodes a protein of 714 amino acids and the deduced protein sequence shares 51% and 21% identity with *T. cruzi* (TcPI-PLC) and *Saccharomyces cerevisiae* PI-PLC, respectively. Southern blot analysis suggested that *TbPIPLC* is present as a single copy gene in the *T. brucei* genome. Northern blot analysis showed a single ~3.2 kb transcript in both bloodstream (BF) and procyclic (PF) forms. Analysis of the ~3.2 kb bands by densitometry indicated that the transcript levels of *TbPIPLC* are similar in both stages. *TbPI-PLC* contains a typical *N*-myristoylation consensus sequence consisting of 18 amino acids at the amino-terminal end. To determine which amino acids of the *TbPI-PLC* *N*-terminus sequence are required for proper targeting to the plasma membrane, we did gene fusions with the *N*-terminus sequences coding for the first 18 amino acids of *TbPI-PLC* or variants in which the glycine (myristoylation site) and cysteine (palmitoylation site) were mutated by site-directed mutagenesis to alanine. These sequences were fused to the green fluorescence protein (GFP) sequence in the *T. brucei* expression vector pUB39. Localization of fusion proteins was observed in transfected parasites using fluorescent microscopy. The wild type 18aa-GFP fusion protein localized to the plasma membrane and flagellum of both BF and PF, Δ C4A localized to the membrane fraction, and Δ G2A localized to the cytosol. Overexpression of the Δ C4A mutant changed the morphology of the parasites and resulted in cell death. We also investigated the importance of *TbPI-PLC* for growth by using RNA interference. When production of double-stranded RNA was induced by tetracycline, growth of PF was not affected. Taking together these results suggest that the first 18 amino acids of the *N*-terminus, and Gly 2 are essential for targeting of *TbPI-PLC* to the plasma membrane, and that the enzyme is not essential for the *in vitro* growth of the PF of *T. brucei*.

14 A FUNCTIONAL AQUAPORIN CO-LOCALIZES WITH A VACUOLAR PROTON PYROPHOSPHATASE TO ACIDOCALCISOMES OF *Trypanosoma cruzi*

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Water channels or aquaporins (AQPs) are intrinsic membrane proteins that allow water or small specific solutes to pass unhindered, but block the passage of ions to prevent dissipation of the transmembrane potential. Here, we report the cloning and sequencing of a gene encoding an aquaporin of *T. cruzi* (TcAQP). The *TcAQP* gene encodes a protein that has 40-55% similarity to other AQPs. The *T. cruzi* protein contains an apparent *N*-terminal signal sequence of 30 aa, which is very similar to the *N*-terminal sequence present in *T. cruzi* V-H⁺-PPase, and has potential *N*-glycosylation and myristoylation sites. The *N*- and *C*-terminal halves of TcAQP have related sequences and each has the signature motif Asn-Pro-Ala (NPA), that is involved in the formation of a single aqueous channel spanning the bilayer. A hydropathy plot showed that, by analogy to human AQP1, TcAQP has six putative transmembrane domains. Southern blot analysis suggested that *TcAQP* is present as a single copy gene. Northern blot analysis showed a single 1.35 kb transcript expressed at similar levels in all *T. cruzi* stages. To investigate the localization of TcAQP we made use of an epimastigote cell line transfected with a green fluorescent protein (GFP)-TcAQP fusion protein (GFP fused at the C-terminal end of TcAQP). Immunofluorescence microscopy showed co-localization in acidocalcisomes of GFP-TcAQP with the V-H⁺-PPase detected with monoclonal antibodies prepared against a conserved epitope in the *T. cruzi* enzyme. In addition there was an anterior localization of TcAQP, close to the flagellar pocket, that was distinct from the acidocalcisomes. The water transport properties of TcAQP were analyzed by expressing the gene in *Xenopus* oocytes and performing oocyte swelling assays. TcAQP-expressing oocytes swelled under hyposmotic conditions indicating water permeability, which was abolished after preincubating oocytes with the AQP inhibitor HgCl₂. These results suggest that TcAQP could play an important role in osmoregulation in *T. cruzi*.

15 INHIBITION OF *Trypanosoma cruzi* FARNESYL PYROPHOSPHATE SYNTHASE BY BIPHOSPHONATES

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Farnesyl pyrophosphate synthase (FPPS) plays a central role in metabolism through the enzymatic generation of farnesyl pyrophosphate, which is used for protein prenylation, and for the synthesis of sterols, dolichols, hemo α , and ubiquinone, and is potently inhibited by bisphosphonates. Bisphosphonates are pyrophosphate analogs in which the oxygen bridge between the two phosphorus atoms has been replaced by carbon substituted with various side chains. Several bisphosphonates are potent inhibitor of bone resorption and

are in clinical use for treatment and prevention of osteoporosis, Paget's disease, hypercalcemia caused by malignancy, and tumor metastases in bone. Bisphosphonates have also recently been shown to be active both *in vitro* and *in vivo* against *Trypanosoma cruzi*, the causative agent of Chagas' disease, without apparent toxicity to the host cells. Moreover, RNA interference and *in vivo* inhibition studies in *T. brucei* has demonstrated that this enzyme is essential for parasite viability and validated this enzyme as a target for drug development (Montalvetti, et al., *J. Biol. Chem.* 278, 2003, *in press*). The recombinant enzyme from *T. cruzi* was shown to be potently inhibited by nitrogen-containing bisphosphonates, while a non-nitrogen-containing bisphosphonate (etidronate) was far less inhibitory, as found with the human enzyme (Montalvetti, et al., *J. Biol. Chem.* 276:33930-33937, 2001). Here, we report the ability of sixteen new compounds to inhibit the *T. cruzi* enzyme.

16 PRESENCE OF A CONTRACTILE VACUOLE IN *Trypanosoma cruzi* AND FUNCTIONAL COUPLING TO ACIDOCALCISOMES

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Trypanosoma cruzi has the ability to recover its cell volume after an hyposmotic challenge by a process termed the regulatory volume decrease. Efflux of amino acids and other osmolytes from the cytosol to the extracellular space can account for 50% of this volume reduction. In many protozoal organisms, a contractile vacuole is a prominent osmoregulatory organelle, and in *Dictyostelium discoideum* and *Chlamydomonas reinhardtii*, mass-dense granules analogous to acidocalcisomes appear to be a distal element of the contractile vacuole complex (Marchesini et al, *JBC* 277:8146-53, 2002; Ruiz et al, *JBC* 276:46196-203, 2001). Therefore, in this work we: (1) present morphological evidence for the presence of a contractile vacuole in *T. cruzi* and its association with acidocalcisomes, (2) provide immunofluorescence microscopy and immunocytochemical evidence for the localization of aquaporin to acidocalcisomes and the contractile vacuole, (3) isolate and partially characterize the contractile vacuole complex and demonstrate the presence of the classical contractile vacuole markers alkaline phosphatase, vacuolar H⁺-ATPase, and calmodulin, and (4) show that aquaporin-containing acidocalcisomes traffic towards and fuse with the contractile vacuole during hyposmotic stress in a cAMP- and microtubule-dependent fashion.

17 MOLECULAR CHARACTERIZATION OF *Trypanosoma brucei* PLASMA MEMBRANE P-TYPE PROTON ATPase

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The ability of *T. brucei* to regulate its intracellular pH (pH_i) and plasma membrane potential ($\Delta\Psi$) throughout its life cycle is critical to its survival, as most intracellular enzymes function optimally within a narrow pH range and the plasma membrane potential is necessary to drive the movement of ions across the membrane. Previous studies (VanderHeyden et al., *Biochem. J.* 346, 53-62, 2000; VanderHeyden and Docampo, *J. Eukaryot. Microbiol.* 49, 407-413, 2002) have demonstrated that a plasma membrane proton pump plays a significant role in the regulation of pH_i and $\Delta\Psi$ in procyclic (PF) and bloodstream (BF) trypomastigotes of *T. brucei*. In the present study, we report the cloning and sequencing of a pair of genes linked in tandem (*TbHA1* and *TbHA2*) in *T. brucei* which encode proteins with homology to the *T. cruzi* P-type H⁺-ATPases (Luo et al., *J. Biol. Chem.* 277, 44497-44506), and fungal and plant H⁺-ATPases. *TbHA1* and *TbHA2* have open reading frames of 2,739 and 2,718 bp, predicted to encode proteins of 912 and 905 amino acids, with molecular masses of 100.2 and 99.5 kDa, respectively. *TbHA1* and *TbHA2* have 76% identity and 85% similarity to the H⁺-ATPases from *T. cruzi*. Northern blotting analysis showed that *TbHA2* is more abundant in BF than in PF stages. Indirect immunofluorescence analysis using antibodies against the *T. cruzi* H⁺-ATPase showed strong labeling of the cell surface in both stages and labeling of intracellular compartments of BF. The essential nature of *TbHA1* and *TbHA2* was studied using RNA interference to inhibit the expression of the genes. Expression of *TbHA1* and *TbHA2* double stranded RNA in both PF and BF caused degradation of mRNA, declination of growth and cell death. Taking into account that plasma membrane P-type H⁺-ATPases are absent in mammalian cells, and that they are essential for parasite viability, these results validate these enzymes as targets for drug development.

18 MOLECULAR CLONING AND CHARACTERIZATION OF PLASMA MEMBRANE-TYPE Ca²⁺-ATPases OF *Trypanosoma brucei*

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The adaptation and survival of *Trypanosoma brucei* in their hosts involves integrated regulation of Ca²⁺ pumps (Ca²⁺-ATPase) which are essential in calcium ion homeostasis. *T. brucei* maintains 4–6 orders of magnitude less cytosolic [Ca²⁺]_{ii} than mammalian host blood milieu. This fact implies that trypanosomes require a Ca²⁺ efflux mechanism to maintain Ca²⁺ homeostasis. Here we report the cloning and sequencing of two genes encoding a plasma membrane-type Ca²⁺-ATPase (PMCA) of *T. brucei* (*TbA1* and *TbA2*), an agent of African trypanosomiasis (sleeping sickness). The proteins predicted from the nucleotide sequence of the genes have 1106 and 1080 amino acids and molecular weights of 121.7 and 119kDa, respectively. *TbA1* exhibits a 94% identity to *TbA2*. A BLASTP search of protein databases shows that *TbA1* and *TbA2* are closely related to plasma membrane-type Ca²⁺-ATPase (PMCA), with a 36% identity at the amino acid level to human PMCA. It also has a 53%, 37%, 32%, and 29% identity with vacuolar Ca²⁺-ATPases of *T. cruzi*, *D.*

discoideum, *T. gondii*, and *S. cerevisiae*, respectively. A hydropathy profile of TbA1 and TbA2 suggests ten transmembrane domains. Indirect immunofluorescence analysis indicates that TbA1 and TbA2 localize to the plasma membrane and co-localize with the vacuolar H⁺-pyrophosphatase to intracellular vacuoles identified morphologically and by X-ray microanalysis as the acidocalcisomes. Northern blot and Western blot analyses revealed that *TbA1* and *TbA2* are upregulated during blood stages but down regulated during culture procyclic stages. *TbA1* and *TbA2* suppress the Ca²⁺ hypersensitivity of a mutant of *S. cerevisiae* that has a defect in vacuolar Ca²⁺ accumulation. *T. brucei* Ca²⁺-ATPase genes were functionally characterized by using double-stranded RNA interference (RNAi) methodology to produce inducible Ca²⁺-ATPase-deficient procyclic strains. The induction of dsRNA (RNAi) caused specific and dramatic loss of the *TbA* mRNA resulting in a FAT cell phenotype, and growth inhibition.

19 FARNESYL PYROPHOSPHATE SYNTHASE OF *Toxoplasma gondii* IS A POTENTIAL DRUG TARGET FOR BISPHOSPHONATES

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Toxoplasmosis caused by *Toxoplasma gondii* (*T. gondii*) can have serious consequences in immunocompromised patients (e.g. AIDS patients) and the developing fetus. The existing therapy for toxoplasmosis is not adequate due to severe adverse side effects and the high cost. To search for effective, non-toxic, and inexpensive chemotherapeutic agents, we focused our studies on the effects of a class of clinically used compounds, bisphosphonates, and the mechanism of inhibition of *T. gondii* by them. The potential target of bisphosphonates in *T. gondii* is the farnesyl pyrophosphate synthase (FPPS), an enzyme that has been found to be the molecular target of bisphosphonates in several other organisms. We have measured and calculated the IC₅₀s of some bisphosphonates against *T. gondii* *in vitro*, which are under investigation by QSAR studies. Some of them show promising inhibition of *T. gondii* growth *in vitro*. In addition, we have cloned and sequenced the gene encoding the *T. gondii* FPPS (*TgFPPS*), and our project is focused on demonstrating that the protein TgFPPS is the target for bisphosphonates. This *TgFPPS* gene contains a 1815-bp open reading frame, encoding a 605-amino-acid protein with a calculated molecular mass of 64.5 kD. The deduced polypeptide sequence of *TgFPPS* gene shares 40% identity and 56% similarity with *Gallus gallus* (chicken) FPPS and 25% identity and 38% similarity with *Trypanosoma cruzi* FPPS. Seven conserved amino acid motifs known to be characteristic of FPPS are also found in the TgFPPS. The alignment of the nucleotide sequence of the *TgFPPS* gene with that of the *TgFPPS* cDNA shows that this gene consists of six introns and seven exons. The results of Southern analyses indicate that the gene encoding TgFPPS exists as a single copy in the genome of *T. gondii* RH.

20 **TgSRCAP: A CHROMATIN REMODELING HOMOLOGUE IN *Toxoplasma gondii***

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Transcriptional regulatory pathways in apicomplexan parasites are understudied and may contain novel drug targets. We have identified and mapped a gene in the opportunistic protozoan pathogen *Toxoplasma gondii* that encodes a homologue of SRCAP (Snf2-related CBP Activator Protein), a member of the SNF/SWI family of chromatin remodeling factors that use ATP to promote a more favorable environment for transcription (known as *domino* in *Drosophila*). The genomic locus (named TgSRCAP) is a single copy containing multiple introns, and the predicted cDNA encodes a protein with features consistent with SRCAP. We have identified additional SRCAP-like sequences in Apicomplexa by screening genomic databases. An analysis of SRCAP homologues between species reveals signature features that we propose may be indicative of SRCAP members. Previous studies show TgSRCAP is functionally analogous to human SRCAP in the sense that it can specifically increase CREB-mediated transcription, and that expression of mRNA encoding TgSRCAP is upregulated during *in vitro* bradyzoite differentiation, collectively suggesting a possible role for TgSRCAP in parasite development (see Reference). Our attempts to “knockout” the TgSRCAP locus by homologous recombination have been unsuccessful, implying that TgSRCAP is an essential gene critical to parasite survival. To further characterize TgSRCAP, we have generated polyclonal antisera to two regions of the protein.

21 **CHARACTERIZATION OF TGGCN5: A NOVEL HISTONE ACETYLTRANSFERASE IN THE PROTOZOAN PARASITE *Toxoplasma gondii***

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Toxoplasma gondii is an obligate intracellular parasite in phylum Apicomplexa. *T. gondii* threatens neonates and immunocompromised (AIDS) patients. *T. gondii* is also recognized as a model for the study of other apicomplexans of importance such as *Plasmodium* spp. (malaria) and *Cryptosporidium* spp. While current therapies effectively treat the invasive form of *T. gondii* (tachyzoite) infection, these drugs are highly toxic and do not eradicate the encysted form of the parasite (bradyzoite). Therefore, novel therapeutics are needed that exert less toxicity and/or target bradyzoites. Apicidin, a novel antiprotozoal agent that interferes with histone modification illustrates that the transcriptional regulatory circuitry may contain original targets for drug research. Previously, our group has cloned a histone acetyltransferase (HAT) homologue in *T. gondii* that resembles GCN5 but possesses unusual features (TgGCN5). In particular, a unique

820 amino acid *N*-terminal extension of unknown function lies upstream of the catalytic domain. The *N*-terminal domain shares no homology to analogous domains found in higher eukaryotic GCN5 proteins, nor is it similar to any sequence in the protein database. Our hypothesis is the *N*-terminal extension of TgGCN5 is critical to the function of this HAT. Three roles for the *N*-terminal domain that are being investigated include: nuclear localization, protein-protein interactions, and regulation of enzymatic function.

22 THE SPATIAL DISTRIBUTION OF URINARY SCHISTOSOMIASIS INFECTIONS AMONG HOUSEHOLDS IN A HIGH PREVALENCE VILLAGE ON THE SOUTHERN COAST OF KENYA

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Geographic information systems (GIS) in conjunction with coarse resolution remote sensing data have been applied to study schistosomiasis in recent years; however, the use of fine resolution satellite data and the application of spatial statistics have yet to be applied until now. *Schistosoma hematobium*, the parasite that causes urinary schistosomiasis is highly endemic in the Msambweni District on the southern coast of Kenya. Demographic and case data for 302 households from one village were collected and mapped. In addition, data for all local water bodies where human contact occurs were collected including location, host and non-host snail numbers, and proportion of snails shedding human cercariae. The village of Milalani was found to be saturated with *S. hematobium* infection with 70% of all school children infected and a mean household prevalence of 36%. Global spatial statistics were used to test whether houses with high infection prevalence were clustered near a water contact site with the highest number of host snails shedding human cercariae.

23 TRYPTOPHAN METABOLISM IN MOSQUITOES WITH EMPHASIS ON 3-HYDROXYKYNURENINE TRANSAMINASES MEDIATED 3-HYDROXYKYNURENINE TO XANTHURENIC ACID

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In mosquitoes, oxidation of tryptophan by tryptophan 2,3- dioxygenase (TDO) to kynurenine is the major pathway of tryptophan catabolism. Kynurenine, once formed, can be converted to kynurenic acid by kynurenine aminotransferase (KAT) or hydroxylated to 3-hydroxykynurenine (3-HK) by kynurenine monooxygenase. Kynurenic acid functions as a broad-spectrum antagonist at ionotropic excitatory amino acid receptors, which protects the

central nervous system from being over-stimulated by excitatory cytotoxins. In contrast, 3-HK is oxidized easily under physiological conditions, stimulating the production of reactive oxygen species. Because kynureine to 3-HK is the major pathway, mosquitoes must have a mechanism to dispose of 3-HK. Our data suggest that mosquitoes control the level of 3-HK by efficient conversion of the chemically reactive and potentially toxic 3-HK to chemically stable xanthurenic acid (XA) through a specific 3-HK transaminase (HKT) catalyzed reaction. Interestingly, 3-HK also is the initial precursor for the production of eye-pigment in mosquitoes during pupal and adult development and coincidentally, HKT is down regulated during pupal and adult stages to allow 3-HK to be transported into the compound eyes for eye-pigmentation. Our results indicate that both KAT and HKT and their molecular regulation play critical roles in regulating the tryptophan metabolism in mosquitoes during development.

24 SEPARATION AND IDENTIFICATION OF MOSQUITO CHORION PROTEINS THROUGH TWO-DIMENSIONAL ELECTROPHORESIS AND MASS SPECTROMETRY

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The chorion or eggshell of insects has extraordinary mechanical and physiological properties. It protects the oocyte or developing embryo from a series of environmental hazards such as drought, temperature variations, mechanical pressure, protease attack, bacterial and viral infections, etc. Proteins are the major component of the mosquito chorion, so knowledge concerning the proteins involved in chorion formation and hardening is essential towards understanding the overall process and mechanism leading to the formation of a protective chorion in mosquitoes. 2-dimensional electrophoresis (2-DE) is the most powerful tool to separate the proteins and in this study, we describe the separation and identification of chorion proteins through 2-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) techniques. Due to their high hydrophobicity, chorion proteins are difficult to solubilize and absorb into immobilized pH gradient (IPG) strips for isoelectric focusing (IEF). By optimizing the applied conditions for chorion protein extraction and sample application, we were able to solubilize a majority of chorion proteins and resolve them through 2-DE. Under the optimized conditions, more than 700 protein spots were resolved by 2-D analysis. Trypsin digestions of individual protein spots, MALDI-MS analysis of their digested peptides, and subsequent blast search of peptide masses resulted in the tentative identification of 38 protein spots. Our data show that sequential extraction of the isolated chorion, 2-dimensional electrophoresis of the solubilized chorion proteins, in-gel digestion of the resolved protein and MALDI-MS analysis of the protein digests is an effective overall strategy towards the determination of chorion proteins in mosquitoes. The merits of the described method for the determination of mosquito chorion proteins and its feasibility for the separation and identification of membrane proteins or eggshell proteins from other insect species are discussed.

25 HUMAN PLATELETS CONTAIN ORGANELLES SIMILAR TO ACIDOCALCISOMES OF TRYPANOSOMATID AND APICOMPLEXAN PARASITES

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Acidocalcisomes are acidic organelles containing pyrophosphate (PP_i), polyphosphate (polyP), calcium, and other elements, which have been postulated to be involved in intracellular pH and calcium homeostasis, and in osmoregulation. Although acidocalcisomes were initially identified only in unicellular parasites, we recently found them in other unicellular eukaryotes such as algae (Ruiz et al., *J. Biol. Chem.* 276, 46196-46203, 2001), and slime mold (Marchesini et al., *J. Biol. Chem.* 277, 8146-8153, 2002), and in bacteria (Seufferheld et al., submitted, 2003). Platelet dense granules are known to be acidic and to contain PP_i and calcium and we therefore investigated their similarity to acidocalcisomes. Dense granules of human platelets were shown to contain large amount of phosphorus, potassium, and calcium, as determined by X-ray microanalysis of intact unstained preparations. The high phosphorus content was due to the presence of PP_i and short chain polyP (less than 50 residues long). PolyP concentration was 10-20 times higher than that of other mammalian tissues, such as brain, heart, kidney, lung, or liver. Dense granules were purified using methrizamide gradient centrifugation, and showed similar structural characteristics to acidocalcisomes of unicellular eukaryotes. Using Fura-2 loaded cells we found evidence that human platelets possess a significant amount of Ca²⁺ stored in an acidic compartment as indicated by (1) the increase in [Ca²⁺]_i induced by nigericin (a K⁺/H⁺ exchanger), monensin (a Na⁺/H⁺ exchanger), or the weak base NH₄Cl, in the nominal absence of extracellular Ca²⁺ to preclude Ca²⁺ entry; and (2) the effect of ionomycin, a Ca²⁺ releasing ionophore that cannot take Ca²⁺ out of acidic organelles and that was more effective after alkalization of this compartment by addition of nigericin, monensin, or NH₄Cl. This acidic compartment was separate from the thapsigargin-sensitive Ca²⁺ pool and similar to the acidocalcisomal Ca²⁺ pool of unicellular eukaryotes. In conclusion, these results demonstrate that acidocalcisomes and platelet dense granules are chemically and functionally similar suggesting a common evolutionary origin.

26 POLYPHOSPHATE CONTENT AND FINE STRUCTURE OF ACIDOCALCISOMES OF *Plasmodium falciparum*

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Although acidocalcisomes have been well characterized morphologically in other apicomplexan parasites, no such characterization has been done in *Plasmodium* spp. Here, we report that *Plasmodium falciparum* merozoites possess electron-dense organelles rich in phosphorus and calcium, as detected by X-ray microanalysis of intact cells, which are similar to the acidocalcisomes of other apicomplexans, but of more irregular form. In agreement with these results malaria parasites possess large amounts of short and long chain polyphosphate (polyP), which are associated with acidocalcisomes in other organisms. PolyP levels were highest in the trophozoite stage of the parasite. Treatment of isolated trophozoites with chloroquine resulted in a significant hydrolysis of polyP. Taken together, these results provide evidence that acidocalcisomes from *Plasmodium falciparum* do not differ significantly from acidocalcisomes of other apicomplexan parasites.

27 WEST NILE VIRUS MAPPING AND ANALYSIS IN ILLINOIS

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West Nile virus (WNV) has spread across the nation since it was first observed in the New York City in 1999. In 2001, West Nile virus was found in dead birds in only a few Illinois counties. During the following year Illinois topped all states in the number of human cases of WNV, logging over 877 cases and 63 deaths. The counties of Cook and Dupage, in the Chicago region, accounted for about 80% of those cases. In the course of the 2002 season, it spread through the bird, mosquito, horse and human populations, with cases across the state. Spatial analysis and sequential mapping are useful and powerful tools to explore the patterns and processes related to the introduction and spread of a newly introduced disease. The College of Veterinary Medicine GIS and Spatial Analysis Lab and the Spatial Epidemiology Lab helped the Illinois Department of Public Health and Illinois Department of Agriculture with mapping and analysis of the WNV in Illinois during the recent outbreak. We demonstrate here the various mapping approaches taken and a summary analysis of the human case clusters that occurred in the Chicago region.

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SPONSORS

CENTER FOR ZONOSSES RESEARCH

The Center for Zoonoses Research (CZR) was established by the Board of Trustees of the University of Illinois on January 20, 1960. The Center is composed of outstanding scientists from 15 departments and units at the University of Illinois at Urbana-Champaign. The goals of the CZR are:

- Promote collaborative work among the College of Veterinary Medicine and other UIUC faculty as well as faculty from other institutions worldwide in an integrated dynamic program.
- Develop a synthesizing approach to zoonoses and infectious disease research based on the unique expertise in veterinary and medical research from the molecular to the ecosystem level.
- Promote the dissemination of information concerning zoonoses research through the organization of conferences and seminars, publication of brochures, proceedings of meetings and reports of research activities, and press releases.
- Promote the establishment of training grants to attract top graduate students, post-doctoral trainees and visiting scientists.
- Promote collaborative efforts and service to the Illinois Departments of Public Health and Agriculture.
- Promote the interest and awareness from UIUC faculty and administration about ongoing research on infectious diseases and food safety
- Become a recognized research and training center by international organizations.

COLLEGE OF VETERINARY MEDICINE

The College of Veterinary Medicine was founded fifty four years ago, in 1949. Since 1970 it has occupied a 56,500 square-foot companion-animal hospital and clinic, a 71,700 square-foot food-animal hospital, and a 158,000 square-foot basic sciences building. The College operates the Laboratories of Veterinary Diagnostic Medicine, that receives some 12,000 samples yearly. Operational units within the diagnostic laboratory include virology, immunology, parasitology, clinical pathology, toxicology, microbiology, and pathology. Further access to veterinary medical accessions is provided through the veterinary teaching hospital.

A variety of support facilities is available in the College. The College has its own photography, histology and library facilities and houses the Center for Microscopic Imaging. The College of Veterinary Medicine Library has 700 specialty serial titles and 32,000 reference books and is part of the University of Illinois library system. The Biomedical Communications Center is available to aid in planning and producing communication materials in art, photography, and television to support research and education. The 12,000

square-foot laboratory animal care facility is designed to meet the needs of research in both noninfectious and infectious diseases. Other animal care facilities, including a 75-acre farm, a fully-equipped companion- and food-animal hospital, and isolation and conditioning quarters, are readily available. The Office of Continuing Education and Public Service offers a variety of consultative services to companion- and food-animal practitioners and the general public. It provides a medium for the dissemination of research findings through regular continuing education conferences and publications.

The College also maintains its own Gopher and World-Wide Web server (<http://www.cvm.uiuc.edu/>). These information services provide a two-way street for College faculty, students and staff to access information anywhere in the world via Internet, and for people outside of the College to learn about College resources and programs.

DEPARTMENT OF VETERINARY PATHOBIOLOGY

The Department of Veterinary Pathobiology plays a central role in the University of Illinois's three-part mission of teaching, research and service. In this land-grant research university, our educational mission is pursued in concert with our research mission. The department encompasses the disciplines of epidemiology and preventive medicine, microbiology and immunology, parasitology, and comparative pathology (<http://www.cvm.uiuc.edu/vp/>).

The majority of departmental research addresses two critical global issues: infectious diseases and environmental toxicology. Both are relevant to human and animal health and have a significant impact on global economic trade. There is extensive collaboration across basic science and clinical departments within the College, across campus, and nationally and internationally. Faculty participates in campus-wide interdisciplinary programs, including those in nutritional sciences, neurosciences, cell and molecular biology, genetics, and environmental toxicology. Support for research comes from the National Institutes of Health, the Centers for Disease Control and Prevention, the U.S. Department of Agriculture, the Illinois Department of Agriculture, the American Heart Association, the World Health Organization, and many other sources.

Departmental research in infectious diseases is coordinated through the Center for Zoonosis Research, which addresses diseases of local, national, and global importance. Major research areas include food safety (diseases due to *E. coli*, *Salmonella* sp., and *Toxoplasma gondii*), vector-borne diseases (malaria, trypanosomiasis, leishmaniasis, Lyme disease, rickettsial diseases, and arbovirus), AIDS-related diseases (cryptosporidiosis, and candidiasis), and animal diseases of economic importance (including those due to *E. coli*, *Salmonella* sp., pseudorabies, rotavirus, porcine reproductive and respiratory syndrome [PRRS] virus, and parasites).

The department has a strong graduate program that attracts students with a B.S., M.S., or D.V.M. degree. Graduate students participate in both the teaching and research functions of the department. The ultimate goal of the graduate program is to produce leaders in biomedical research and education for the 21st century. Residency programs in pathology and continuing education courses, such as molecular biology, are also offered. Undergraduate teaching is increasing through courses taught collaboratively with other

departments and through research opportunities within the department. Computer technologies are used to enhance instruction and develop distance learning, which is critical to education today and in the future.