Human Pathogens within the Dimorphic Onygenales
Asilomar Grounds: Triton

Please come directly to Triton between 9 and 9:30 AM for registration

9-9:30 Registration
9:30-9:45 Opening Welcome Address. Bridget Barker, Translational Genomics Research Institute’s Pathogen Genomics Division (TGen North)
9:45-10:15 John Taylor, UC Berkeley. Natural History of Coccidioides
10:15-10:45 Gregory Gauthier, Univ. of Wisconsin. Molecular regulation of dimorphism in Blastomyces dermatitidis
10:45-11:15 Coffee Break
11:15-11:45 Meritxell Riquelme, CICESE. Molecular detection of Coccidioides spp. in Baja California.
11:45-12:15 Anita Sil, UCSF. Title TBA (Histoplasma capsulatum)

12:15-12:30 Discussion

12:30-2:00 Lunch break (Crocker dining hall for those who purchase meal plan or lunch ticket, or lunch offsite)

2:00-2:30 Jonathan Finkel, BEI Resources, NIAID’s BEI Resources, Providing Reagent Support for Research on Pathogenic Fungi
2:30-2:45 Chelsea Boyd, UNC, Discovering Histoplasma factors required for initial macrophage interaction
2:45-3:00 Bevin English, UCSF, Manipulation of macrophage biology by the intracellular fungal pathogen Histoplasma capsulatum
3:00-3:15 Sinem Beyhan, UCSF, Histidine kinase pathway components are required for growth in the parasitic form of Histoplasma capsulatum

3:15-3:30 Coffee Break

3:30 ~ 4:30 Panel Discussion

6:00 Opening of 2015 GSA Fungal Genetics meeting
ABSTRACTS

Dimorphism, lipids, and a GATA transcription factor in *Blastomyces dermatitidis*

Gregory Gauthier, UW

In response to temperature, *Blastomyces dermatitidis* converts between yeast and mold forms. Knowledge of the mechanism(s) underlying this response to temperature remains limited. In *B. dermatitidis*, we identified a GATA transcription factor, *SREB*, that influences the transition from yeast to mold. Null mutants fail to fully complete the conversion to mold and cannot properly regulate siderophore biosynthesis. To capture the transcriptional response regulated by *SREB* early in the phase transition (0 - 48 hours), gene expression microarrays were used to compare *SREB* to an isogenic wild type isolate. Analysis of the time course microarray data demonstrated *SREB* functioned as a transcriptional regulator at 37°C and 22°C. Bioinformatic and biochemical analyses indicated *SREB* was involved in diverse biological processes including iron homeostasis, neutral lipid biosynthesis, and lipid droplet formation. Integration of microarray data, bioinformatics, and chromatin immunoprecipitation identified a subset of genes directly bound and regulated by *SREB in vivo*. This included genes involved with siderophore biosynthesis and uptake, iron homeostasis, and genes unrelated to iron assimilation. Functional analysis suggested that lipid droplets were actively metabolized during the phase transition and lipid metabolism may contribute to
Molecular detection of *Coccidioides* spp. in Baja California.


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Coccidioidomycosis (Valley Fever) represents a serious threat to inhabitants of endemic areas of North America. Despite successful clinical isolations of the fungal etiological agent, *Coccidioides* spp., the screening of environmental samples has had low effectiveness. We set out to analyze this pathogen in its natural environment to characterize its ecological niche. We have explored an area of Valle de las Palmas (VDP), in Baja California, Mexico, previously predicted as a putative endemic “hotspot”, to detect *Coccidioides* spp. via culture-independent molecular methods. Two different microhabitats, burrows (influenced by rodent activity) and surface (topsoil), were included in the analyses. Initially, a nested PCR designed to amplify the complete internal transcribed spacer (ITS) region of Dikarya followed by a diagnostic PCR designed to amplify the ITS2 region of *Coccidioides* spp. were carried out using the total genomic DNA extracted from soil samples (n=93). While a high number of the recovered amplicons (37) were confirmed to belong to *Coccidioides* spp., other non-specific sequences belonging to *Aphanoascus canadensis*, *A. keratinophilus*, *Penicillium cyclopium* and *P. dipodomyicola* were also amplified. After multiple alignment of the ITS region for all fungal species identified with the ITS2 primers, new primers were designed to amplify the ITS1 region of *Coccidioides* spp. and reduce non-specific amplifications. With the new primers, all the putatively positive amplicons were identified as *Coccidioides* spp. In all cases a higher prevalence of *Coccidioides* spp. was observed for burrow (33) than for topsoil samples (15). In addition, by testing the serum from 40 rodents trapped in VDP with ELISA, we detected antibodies against *Coccidioides* in two species: *Peromyscus maniculatus* (deer mouse) and *Neotoma lepida* (woodrat).

NIAID’s BEI Resources, Providing Reagent Support for Research on Pathogenic Fungi

BEI Resources was established by the National Institute of Allergy and Infectious Diseases to provide cultures, reagents, and information for studying Category A, B, and C priority pathogens, emerging infectious disease agents, non-pathogenic
microbes and other microbiological materials of relevance to the research community. BEI Resources acquires, authenticates, and produces reagents that scientists need to carry out basic research and develop improved diagnostic tests, vaccines, and therapies. Researchers worldwide can register to request any of our catalog materials within their registration level and they will receive the reagent FREE of charge. By centralizing these functions within BEI Resources, quality control of the reagents is assured. In addition to supplying the infectious disease community with materials, BEI Resources also encourages and supports the deposit of materials from researchers and institutions, following NIAID review and approval of the material. Depositing materials with BEI Resources has many advantages to the researcher including secure storage, community access and distribution; while protecting the material rights of the depositor and institution. Use of the BEI Resources program ensures a long term commitment to enabling future research with key, well-characterized pathogenic strains and reagents.

Discovering Histoplasma factors required for initial macrophage interaction

Chelsea D. Boyd, Tracy Carlson, Victoria E. Sepúlveda, and William E. Goldman
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Histoplasma capsulatum, is a dimorphic fungal pathogen that undergoes a temperature-induced transition from a mold that grows in the soil, to a parasitic yeast form that establishes infection in the lung and is capable of causing severe systemic disease in mammals. H. capsulatum is a parasite of macrophages, proliferating intracellularly in a membrane-bound compartment of relatively neutral pH. The ability to modulate and survive within the hostile environment of the macrophage is integral to H. capsulatum pathogenesis. In order to promote and sustain its intracellular survival, we predict that H. capsulatum likely initiates these processes via specific cell surface factors that trigger selective uptake by macrophages.

We employ a random mutagenesis approach to identify factors employed by H. capsulatum that are required for initial macrophage attachment. A library of ~50,000 H. capsulatum Agrobacterium T-DNA-insertional mutants was generated. H. capsulatum mutants are enriched and screened to yield several candidates containing mutations in genes required for macrophage attachment. Targeted gene disruption and complementation assays will verify the requirement of specific genetic elements for virulence in vitro and in vivo. The completion of this project will uncover molecular determinants required for H. capsulatum attachment to macrophages and yield a fundamental understanding of the initial interaction required by H. capsulatum for the establishment of a successful infection of macrophages.

Manipulation of macrophage biology by the intracellular fungal pathogen Histoplasma capsulatum. Bevin English, Young Nam Lee, Dervla Isaac,
Histoplasma capsulatum (Hc) is a primary fungal pathogen of humans and other mammals. As an intracellular pathogen, Hc is able to subvert the immune function of naïve macrophages and replicate within the phagosome, eventually causing macrophage lysis. However, the mechanism by which Hc causes host-cell death is unknown. Macrophage lysis is dependent upon the secreted protein Cbp1 (calcium binding protein 1, ref 1); we show that cbp1 mutant yeast are able to grow to high levels within macrophages, but these host cells do not lyse, indicating that high intracellular fungal burden is not sufficient to trigger host-cell death. Because Cbp1 has no known protein domains and only a few orthologs which are relatively unstudied, we undertook two exploratory approaches to begin to elucidate the mechanism by which Cbp1 mediates the interaction between Hc and its host. The first approach is a comprehensive alanine scanning mutagenesis of Cbp1, which enabled us to identify a group of acidic residues at the N terminus that are necessary for macrophage lysis. We are currently assessing the mutant library for other Cbp1 properties, such as calcium binding, which will enable us to either link or uncouple these properties to the ability to trigger host-cell death. The second exploratory approach was transcriptional analysis of infected macrophages, which led to the identification of a set of host genes that are induced during Hc infection in a Cbp1-dependent manner. Several of these host genes are involved in ER stress and apoptosis, including Tribbles3 (TRB3). Here we show that macrophages deficient for TRB3 are resistant to Cbp1-mediated lysis. Similarly, macrophages lacking both Bax and Bak, key components of the apoptotic pathway, are also resistant to lysis during Hc infection. These data suggest that Cbp1 induces apoptosis in the host cell, and we are currently investigating host factors that interact with Cbp1 to cause macrophage death.
diverse set of proteins that interact with Ryp2 and Ryp3, we characterized two Ryp2-interacting proteins with predicted response regulator domains. Response regulators and sensor histidine kinases form two-component regulatory systems that are often involved in sensing environmental signals using a phosphorelay mechanism. Because it was previously shown that a sensor histidine kinase, Drk1, is required for the yeast-phase growth (Nemecek et al 2006 Science), we hypothesized that the two Ryp2-interacting response regulators could also be important for yeast-phase growth in *H. capsulatum*. Confirming our hypothesis, knockdown of these two genes (named *RYP5* and *RYP6*) resulted in filamentous growth regardless of temperature, indicating that they are required for yeast-phase growth. Additionally, from our previous studies, we found that *RYP5* and *RYP6* are not regulated (directly or indirectly) by Ryp1-4, suggesting that Ryp5 and Ryp6 may act upstream of Ryp1-4. We are currently investigating whether Ryp5 and Ryp6 act together with Drk1 and upstream of Ryp1-4 to regulate yeast phase growth. These experiments are highly significant since they will reveal the importance of two-component regulatory systems for sensing host temperature and provide a molecular understanding of how a pathogenic fungus responds to host temperature to cause disease.