***{for Promotion to Professor tenured}***

**JOHN C. PANEPINTO, PH.D.**

**Associate Professor of Microbiology and Immunology**

**Personal Statement**

**Statement on Research and Creative Activity.** There are estimated to be more than one million species of fungi on the planet, yet very few of these species are capable of causing disease in humans. Of those that can cause systemic infections in humans, there are closely related species that cannot, despite high similarity in virulence factor production and in the complement of genes in their genomes. If there are not unique genes or virulence factors that allow one organism to be a pathogen and one not, then what is allowing this distinction? I would purport that this distinction arises from a differential ability to respond to stress and make necessary adaptations to survive and overcome the stress condition, i.e., a re-wiring of existing regulatory pathways. The fundamental question that we are addressing in my laboratory is how the fungal pathogen, *Cryptococcus neoformans*, adapts from a comfortable environmental temperature to the stress-inducing temperature of the host. We are approaching this problem from the perspective of regulation of mRNA, the intermediate messenger between the gene itself and the functional protein. Recent advances in our understanding of gene expression suggest that the regulation of mRNA fate, also called post-transcriptional gene regulation, plays a fundamental role in regulating the expression level of a gene.

We have demonstrated that the process of mRNA degradation is fundamental to the ability of *C. neoformans* to adapt to the temperature of the human host, and therefore, to cause disease. A related *Cryptococcus* species, *C. amylolentus*, is similar to *C. neoformans* in environmental distribution and is able to cause disease in a surrogate host, the waxworm *Galleria mellonella,* at 25oC, lacks the post-transcriptional reprogramming present in the human pathogen, and is therefore avirulent in humans and animal models. Using *C. amylolentus* as a tool, we are currently dissecting the molecular mechanisms that allow *C. neoformans* to adapt to host temperature through post-transcriptional reprogramming. This work is currently under revision at *Nature Communications*. Included in this manuscript is an RNA-seq analysis of total mRNA and ribosome-associated mRNA in the wild type and deadenylation-deficient mutant of *C. neoformans.* This analysis revealed that a failed reprogramming of the translating mRNA pool that also indicates effects on transcriptional reprogramming, rendering the pathogen incapable of adapting to its host. These results suggest that in order for the organism to adapt, it must first purge itself of the tools for rapid growth in order to synthesize the tools required for pathogenesis.

In addition to investigating the molecular mechanisms of host temperature adaptation, my lab has recently begun investigating the consequences of impairing temperature adaptation on host-pathogen interactions. Impairing mRNA decay in response to host-temperature leads to exposure of pathogen-associated molecular patterns (PAMPS) that are normally masked in wild type *C. neoformans.* One such PAMP is b-1,3-glucan, a known ligand for the Dectin-1 pattern recognition receptor. Through collaboration with Dr. Elizabeth Wohlfert, we have determined that exposure of b-1,3-glucan leads to increased phagocytosis of our mRNA decay mutant by resident alveolar macrophages in mice, leading to rapid clearance. We have submitted an R21 to National Institute of Allergy and Infectious Disease (NIAID) to expand this work to investigate both *C. neoformans* pathways governing antigen masking, as well as to define the alterations to the pulmonary immune landscape when b-1,3-glucan is exposed on *C. neoformans.* I am excited for the potential to identify small molecule antifungal therapies that could induce antigen unmasking, allowing the innate immune system to be activated to clear *C. neoformans*, which could improve outcome in patients with defects in adaptive immunity, such as those with AIDS.

The investigation into the translating pool of mRNA has led us to look more closely at the fungal ribosome. The ribosome is a vetted target for anti-bacterial therapies, but the presumed similarities between the fungal and human ribosome has made exploitation of the ribosome as a drug target difficult. Work performed by Jay Leipheimer in my lab during his doctoral work has looked specifically at stress-responsive translation, and demonstrated that the major regulator of stress responsive translation, the eIF2-a kinase Gcn2, is required for oxidative stress resistance in *C. neoformans (manuscript in preparation)*. In addition, thanks to proteomics seed funding through the UB VPR office, we have identified *C. neoformans*- and fungal-specific factors that associate with the ribosome during stress. These newly identified translation-associated factors will be investigated in future work as potential avenues for exploiting the fungal ribosome as an antifungal target.

A parallel focus of my lab in the last several years has been investigating the molecular mechanism of intrinsic resistance of *C. neoformans* to the antifungal drug caspofungin. Work begun on post-translational regulation of RNA binding proteins by protein arginine methyltransferases has revealed that the Rmt5 is required for intrinsic caspofungin resistance, and that an RNA binding protein, Puf4, which contains a putative methylation site, is a negative regulator of caspofungin resistance. The mRNA encoding the target of caspofungin, the b-1,3-glucan synthase *FKS1*, is destabilized in a *puf4*D mutant, leading to increased intrinsic resistance, suggesting that intrinsic caspofungin resistance is regulated post-transcriptionally. A Ph.D. student, Murat Can Kalem, is currently following up on these studies, and the role of protein arginine methylation in *C. neoformans* antifungal drug susceptibility and pathogenesis.

In summary, my lab is taking a unique approach to studying *C. neoformans* pathogenesis and stress adaptation, as we are the only lab investigating post-transcriptional regulation of gene expression and the fungal ribosome in the context of stress adaptation. Our forward trajectory is to focus on the molecular mechanisms that promote translational reprogramming in *C. neoformans* in response to stress. Major questions that we will address in future work include the following: 1. What mechanism confers specificity to mRNA degradation during temperature adaptation, and are these molecular interactions viable targets for small molecule therapeutics? 2. What signaling pathways promote reprogramming, cell wall remodeling, and antigen masking during stress adaptation, and could targeting these pathways aide clearance in immunosuppressed patients? 3. Are there fungal-specific and *Cryptococcus-*specific translation factors that could be targeted to impair host-adaptation in *C. neoformans*? This trajectory will lead to a comprehensive understanding of the process by which *C. neoformans* reprograms gene expression during the process of stress adaptation, how this reprogramming promotes immune evasion and pathogenesis, and what components of this process might be viable targets for novel antifungal therapies.

**Statement of Service:** Since promotion to Associate Professor, I have had several opportunities to apply my skills and professional knowledge to further the education and research mission of my department, the Jacobs School and UB as a whole. At the departmental level, in addition to the graduate studies roles and curriculum revision roles (see *Statement on Educational Activities and Teaching)*, I have taken on a new role in mentoring junior faculty in the department as part of the departmental mentoring committee.

Beyond service to my department, I have represented my department in a number of capacities in the Jacobs School of Medicine and Biomedical Sciences. I have served a two-year term as the Parliamentarian on the Faculty Council Steering Committee, as well as being a member of the Faculty Council Bylaws committee. As part of the Bylaws Committee, we were instrumental in modifying the committee structure in the Jacobs School to allow more flexibility in committee population and in committee activities. I served as a member of the Jacobs School Research Strategic Plan Committee which developed the current Jacobs School Research Strategic Plan, and I am still involved in the implementation process of the plan and recommended action items, especially those that relate to graduate student recruitment, retention and diversity.

For the last three years, I have been Director of the Witebsky Center for Microbial Pathogenesis and Immunology. In this capacity I have utilized center assets to support the research of the member investigators, have instituted a postdoctoral research exchange seminar series and have hosted several career development events for graduate students and postdocs in the Jacobs School. In addition, I have served the University as a member of the Radiation Safety Committee since 2015.

Beginning in September of 2018, I was appointed Director of Recruiting and Admissions for the Ph.D. Program in Biomedical Sciences (PPBS), which also includes directing the Summer Research Experience (SURE) program for undergraduates. In August of 2018, prior to beginning my Admissions and Recruitment duties, I authored an NSF Research Experience for Undergraduates (REU) proposal that would support an expansion of our SURE program. Although it was not funded on the first submission, the reviews were favorable, and I will resubmit the proposal this coming August. As Director of Admissions and Recruitment, I focused recruitment efforts on primarily undergraduate institutions that are nearby and in NYC. I also made a comprehensive revision in our admissions procedures which included the development of a rubric-based assessment tool for paper application review. Also, with the support of the Senior Associate Dean for Research and Graduate Education and the Basic Sciences Chairs, I reinstituted the practice of interviewing prospective Ph.D. students prior to making offers. This change along with the curricular revision described above are steps forward in presenting a rigorous and truly interdisciplinary graduate program to prospective students. Prospective data collection will allow us to make adjustments to the process as needed. Through my oversight of both the SURE Program and Recruitment for the PPBS program, I hope to collaborate and synergize with existing efforts to attract students to the Jacobs School who will enhance further our diversity, and academic and research excellence.

I have used my knowledge to reach outside of UB to disseminate science to both my profession and to the local community in Buffalo. From 2010 to 2018, I organized the Buffalo Conference on Microbial Pathogenesis, sponsored by the Witebsky Center for Microbial Pathogenesis and Immunology. I represented UB at the Buffalo Museum of Science in a community outreach talking about the human microbiome for the GEM Community of Excellence Science After Hours series. In March of 2019 I was a session chair for the Fungal Genetics Conference sponsored by the Genetics Society of the America.

In service to my scientific community, I have performed Ad Hoc Peer Review Service for the NIH, the American Heart Association, the Agence Nationale de la Recherche (ANR), France, and for the Henry Dale Fellowship. Wellcome Trust, UK. I reviewed fellowships and grants for the American Heart Association as part of the Basic Sciences 2 (BSC2) review panel, and after the recent reorganization, served for one meeting of the predoctoral fellowship committee. In addition, I have served on the Awards Committee for the Medical Mycological Society of the Americas (MMSA). In July of 2019, I will begin a 4-year term as a member of the HIV-associated Cancers and Co-Infections (HCAC) NIH study section. Finally, as part of my role in PPBS admissions and recruitment, I am the Jacobs School representative to the American Association of Medical Colleges (AAMC) Graduate Research, Education and Training (GREAT) Group.

In service to the Greater Western New York Community, I have served on the Long Term Planning Committee for The Niagara Regional Theatre Guild, a community theatre in Tonawanda that has been presenting theatrical productions to the WNY community for 96 years. I also organized benefit concert events under the name Care Cabaret Buffalo that have raised funds for St. Luke’s Mission of Mercy, The American Foundation for Suicide Prevention, Journey’s End Refugee Services, and The Catholic Charities Disaster Relief Fund for hurricane victims in Puerto Rico.

**Statement on Educational Activities and Teaching.** I often use the phrase, “I am a true academic at heart”. My research and scholarly activities are driven by a desire to learn, and I attempt to kindle this same desire as I teach and mentor students whom I engage in the classroom or laboratory. Since beginning at UB in 2008, I have successfully mentored four Ph.D. students and one M.S. student to degree conferral. I am currently mentoring four Ph.D. students in my laboratory.

Since my promotion to Associate Professor, I have taken a more active role in curriculum development for both the Microbiology and Immunology MS and Ph.D. programs, as well as in the revision of the PPBS curriculum. As Director of the Master’s Program in Microbiology and Immunology (2012-2014), I implemented a mechanism for our coursework-based MA students to gain research experience through independent study in PI laboratories. This ensured that students who matriculated through the Master’s program had an opportunity to engage meaningfully with a PI, discern if research as a career was a good fit, and obtain a strong reference for future career development. In 2014, I assumed the role of Director of Graduate Studies (DGS) in Microbiology and Immunology (2014-2016), which coincided with our 5-year program review conducted by the Graduate School. I collaborated with vice-chair Noreen Williams to co-author the self-study document, and was actively engaged in the review, and the revision of the core curriculum that ensued following the receipt of the review recommendations. After my tenure as DGS, I remained active in the departmental graduate affairs committee, and was tasked with pursuing a comprehensive revision of the MS curriculum in collaboration with the Director of the MS program and another faculty member. Through this process, we have developed a MS curriculum that is both rigorous and flexible to allow students to fulfill requirements in three semesters with the option of a fourth semester for thesis work. As part of this curriculum, I developed an MS-only seminar course for the first semester that teaches incoming students how to approach a scientific paper thoughtfully and critically, with the goal of the MS students matching our Ph.D. students in their ability to discuss papers in upper level seminars.

In the fall of 2018, I was appointed as the Microbiology and Immunology representative to the PPBS steering committee where we were tasked with a total revision of the first semester PPBS curriculum. I feel strongly that the committee created an innovative, interdisciplinary curriculum that will serve our incoming PPBS students and the interdisciplinary research environment of the Jacobs School. I submitted our curriculum development plan as a poster presentation for the Graduate Research Education and Training (GREAT) group meeting of the American Association of Medical Colleges (AAMC) to take place in September. We intend to take advantage of the newly created Medical Education Research Institute (MERI) to assist us in prospectively monitoring educational outcomes of our new curriculum.

Finally, I feel strongly that part of my role as a faculty member and mentor is to promote mental health and wellbeing of students in the Jacobs School and UB. To this end, I have been a member of the Student Progress Committee which sees students who have failed two modules in the M1 and M2 years and makes recommendations to help the student correct impediments to success and avoid dismissal. I am also a new member of the Jacobs School Standing Committee on Diversity, Inclusion and Learning Environment, which is tasked with setting policy to ensure that the Jacobs School learning environment is safe, healthy, diverse and inclusive. Finally, within the NSF proposal for the SURE program is the inclusion of a life-skills component to the summer research experience in which students will be taught and encouraged to practice particular coping skills on a weekly basis.