Title – Characterization of a Temperature Sensitive Rescue Mutation in C. *neoformans*Program of Study – Molecular Biology
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Abstract: In order to cause disease, a microbe must procure the basic building blocks of life (e.g. carbon and nitrogen) from its environment. Prior studies by colleagues at the University of British Columbia and Duke University have shown how important carbon acquisition is for microbe virulence throughout the human body, making it a critical topic of continued investigation. We will use routine molecular biology techniques including DNA cloning, PCR, and biolistic transformation to investigate the genetic basis of rescue mutations in the $pykl\Delta$ strain of Cryptococcus neoformans. We have identified several rescue mutants that exhibit growth on glucose as the sole carbon source while lacking a functional pyruvate kinase. Previous Liberty University Research students identified genes potentially responsible for the rescue phenotype from unpublished transcription profiling data, and we have created pLANDO-2, an overexpression construct with CNAG_01268 cloned in behind the histone-3 promoter in pCN19. This overexpression construct will be cloned into $pykl\Delta$ to see if we can recapitulate the rescue phenotype. Additionally, we will attempt to clone other candidate genes (CNAG_01002 and CNAG_01256) into pCN19 and pCN20 vectors; subsequently, transformed $pyk1\Delta$ mutants will be further characterized by evaluating known traits associated with ability to cause disease, such as melanin and capsule production. Through the completion of this project, we will extend our understanding of the role carbon metabolism plays in the virulence of *C. neoformans*, and to potentially exploit carbon metabolism for prevention of disease. These studies will complement and build upon current investigations in Cryptococcus biology and provide a means for collaboration with colleagues at other institutions in the near future.