BCHM 421/422 – 2019/2020

Project Outline:
Candida albicans is a benign inhabitant of the digestive tract and other mucosal surfaces of most healthy individuals, but is associated with significant morbidity and mortality in immunocompromised people. In these instances, it can infect a broad range of niches within the host and frequently acquires resistance to antifungal agents using an assortment of adaptive mechanisms. A major adaptive mechanism involves altering the copy number of genes that improve survival under stress through generation of aneuploidies. To better understand the molecular mechanisms of ploidy changes in this fungus, we aim to identify and characterize the key players involved in mitotic spindle formation and chromosome segregation in *C. albicans*, as these are processes from which aneuploidy can rapidly arise. We will focus on the roles of three kinesin motors that regulate mitotic spindle dynamics in *C. albicans*.

Supervisor: Dr. John Allingham, allinghj@queensu.ca

Project Title: Investigating the roles of kinesin motors in genome plasticity of Candida albicans

Keywords: CANDIDA ALBICANS, ANEUPLOIDY, KINESIN, MITOTIC SPINDLE, MICROTUBULES

Project Goals:
1. Delineate how these kinesins work together to assemble and influence spindle structure during mitosis, and how these activities are regulated during stress conditions to promote aneuploidy
2. Identify binding partner proteins that specify localization and function of each kinesin

Experimental Approaches:
We have a large library of molecular tools and genetic engineering methods, such as a *C. albicans* CRISPR system, to develop mutant *C. albicans* strains. Our lab's expertise in protein expression control and live cell imaging, will enable students to determine the cooperative and/or antagonistic roles of the kinesins in spindle function and chromosome segregation, and learn how these roles may be manipulated by stress-response pathways to alter cell ploidy. The stress conditions will include antifungal agents and non-utilized carbon sources. Students will also learn to perform biochemical and biophysical studies of purified kinesins within reconstituted microtubule-based systems to understand the mechanistic basis for functional changes in kinesin activities leading to mitotic errors and genetic rearrangements.

Impact:
Understanding the pathways that afford *C. albicans* with a higher potential to become drug resistant may lead to improved strategies for preserving the efficacy of existing antifungal agents.

References:
1. Harrison, B. D., Hashemi, J., Bibi, M., Pulver, R., Bavli, D., Nahmias, Y., Wellington, M., Sapiro, G. & Berman, J. (2014). A tetraploid intermediate precedes aneuploid formation in yeasts exposed to fluconazole. *PLoS Biol* **12**, e1001815.
2. Selmecki, A., Forche, A. & Berman, J. (2010). Genomic plasticity of the human fungal pathogen Candida albicans. *Eukaryot Cell* **9**, 991-1008.
3. Selmecki, A. M., Dulmage, K., Cowen, L. E., Anderson, J. B. & Berman, J. (2009). Acquisition of aneuploidy provides increased fitness during the evolution of antifungal drug resistance. *PLoS Genet* **5**, e1000705.
4. Frazer, C., Joshi, M., Delorme, C., Davis, D., Bennett, R. J. & Allingham, J. S. (2015). Candida albicans Kinesin Kar3 Depends on a Cik1-Like Regulatory Partner Protein for Its Roles in Mating, Cell Morphogenesis, and Bipolar Spindle Formation. *Eukaryot Cell* **14**, 755-74.