BCHM 421/422 – 2020/2021

Project Outline:
Fungi are significant and increasing mediators of pathogenesis in people; causing serious challenges medically and economically. *Candida albicans* is the most prevalent cause of fungal infections, and can cause life-threatening systemic infections if our immune defenses are compromised. A battery of fitness attributes promote pathogenicity and drug resistance of this fungus, most of which arise by rapid generation of genetic diversity within a fungal population in response to stressful growth conditions, such as exposure to antifungal drugs, as a means of adaptation. Research has shown that aneuploidy (an abnormal number of chromosomes) accounts for much of this diversity, and that this condition often arises from changes in the activity of certain components of the chromosome segregation machinery. Our findings indicate that some of these changes could be induced or enabled by kinesin motor proteins that regulate the structure of the cell's chromosome segregation apparatus – 'the mitotic spindle'.

Supervisor: Dr. John Allingham

Project Title: Investigating the roles of kinesin motors in stress adaptation by Candida albicans

Keywords: Candida albicans, fungal pathogen, aneuploidy, kinesin

Project Goals:
1. Use X-ray crystallography and cryo-EM to determine the structure-function relationships of kinesins that influence spindle structure during mitosis.
2. Use protein tagging and affinity pull-down assays to identify kinesin binding partner proteins that specify localization and function of each kinesin.
3. Test stress conditions that promote aneuploidy in *C. albicans*, and determine their effect on kinesin activity in cells via fluorescence microscopy.

Experimental Approaches:
Our lab's expertise in protein expression, purification and structure determination will enable students to determine the structure-function relationships of kinesins in Candida. We also have a large library of genetic engineering methods, such as a CRISPR gene editing system, to develop mutant *C. albicans* strains that will help us understand the roles of 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 cooperative and/or antagonistic activities of the kinesins that lead 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. Shoukat, I., Frazer, C. & Allingham, J. S. (2019) Kinesin-5 Is Dispensable for Bipolar Spindle Formation and Elongation in *Candida albicans*, but Simultaneous Loss of Kinesin-14 Activity Is Lethal. *mSphere* **4(6)**: e00610-19.
2. 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.