BCHM 421/422 – 2020/2021

Project Title: Kinesins that control actin polymerization dynamics in cancer cells

Project Outline:
Microtubules and actin filaments form dynamic networks to organize the nucleus and cytoplasm, and to sense and influence cell shape. For example, microtubules grow and shrink to establish the correct size and morphology of the mitotic spindle apparatus for accurate chromosome segregation. Actin filaments are rapidly assembled and bundled together within higher-order structures like the contractile ring to mediate cell division. In both cases, precise temporal and spatial control of their polymeric state is required, but the large differences in their lattice structure and mechanical properties necessitate differential modes of regulation. Our research program is providing a mechanistic understanding of the molecular actions of a unique kinesin motor that promotes actin polymerization and bundling.

Supervisor: Dr. John Allingham

Keywords: Cancer, cell division, actin dynamics, kinesin motors, structural biology

Project Goals:
1. Protein truncation studies and chemical biology methods will be used to identify the actin-binding interfaces of the kinesin.
2. X-ray crystallography and electron microscopy (cryoEM) will be used to determine high-resolution structures of kinesin complexes bound to actin subunits and actin polymers, respectively.

Experimental Approaches:
Crucial to understanding the molecular basis of the kinesin's ability to stimulate actin polymerization and bundling will be to study its interactions with actin. Using a truncation analysis of the kinesin protein, we will identify the minimal actin-binding regions. We will then pinpoint the specific binding residues within these interfaces using a chemical biology method that involves chemically labeling lysines in the kinesin protein. By isolating modified kinesin molecules whose co-sedimentation with actin polymers by ultracentrifugation is disrupted, followed by mass spectrometry profiling of trypsin-digested peptides of these modified kinesin molecules, we will reveal the essential residues. With this information, we will conduct focused mutagenesis to probe for sites that functionally relate the actin-polymerization enhancement activity of this protein. This will be assessed by microscopy methods using fluorescent actin. We will also provide a view of these regions of the kinesin by solving its crystal structure. Furthermore, we will grow crystals of the actin-binding domain of the kinesin in complex with actin subunits that are blocked from polymerization by capping proteins or by using our actin-depolymerizing small molecules. We also have the option of using cryo-EM to view the kinesin's interaction with F-actin polymers.

Impact:
These studies will ultimately lead to a better mechanistic understanding of how the dimensions of actin-based cellular superstructures are dynamically regulated. Personnel trained during this research project will acquire expertise in biochemical and structural biology research methods that are sought-after by academic and industrial research employers.

References:

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