Ramapo Honors Thesis Research Summary

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I have worked with Dr. Monen for the past three years on several different projects which all focus on understanding the process of cell division in the nematode *Caenorhabditis elegans*. During my sophomore year, I assisted in the creation of a live imaging technique for observing mitosis and meiosis in *C. elegans*. I contributed the design for a three-dimensionally printed slide with a large opening in the center. This design allowed easy access to single-cell embryos while they underwent their first mitotic division. This allowed us to further study the role of specific proteins in mitosis. The centromeric protein CENP-A, which is critical for kinetochore deposition and chromosome segregation in mitosis is uncoupled from kinetochore assembly and not required to segregate chromosomes during meiosis. *C. elegans* have 2 CENP-A homologs; HCP-3 and CPAR-1. HCP-3 plays a conserved centromeric role critical for mitotic chromosome segregation, whereas CPAR-1's role has yet to be determined.

Recently, it has been shown that the protein CPAR-1, which localizes to meiotic chromosomes, is cleaved by the cysteine-protease Separase at the metaphase-to-anaphase transition during Meiosis I. Using the nematode *C. elegans*, an RNAi approach coupled with live imaging and immunofluorescence can help to elucidate the role of Separase-mediated cleavage of CPAR-1 in meiosis. The imaging techniques being utilized employ the use of a Zeiss Axiovert 200M epi-fluorescent microscope. Two different protocols have been used to create slides for live imaging of both meiosis and mitosis in wild-type strains of *C. elegans* as well as RNAi injected strains of *C. elegans.* The first technique creates a slide utilizing a KP buffer environment for dissection and an agar pad for slide preparation. The second technique utilizes meiosis media to provide an improved environment for dissection, and a three-dimensionally

printed slide with a system of cover-slips for slide preparation. The second technique has proved to be more successful as the nematode embryos persist long enough to undergo both meiotic and mitotic processes without arresting. This allows for video capture of the first embryonic mitotic division from beginning to end.

During my junior and senior years, I wanted to focus more on the production and utilization of proteins relevant to my previous research experience. I focused specifically on antibodies which have a broad range of research applications in cell biology. Their ability to bind to specific molecules makes them ideal probes for detecting the presence of an antigen and for localization studies inside the cell, which are utilized in our research to study the molecular underpinnings facilitating chromosome segregation. To advance these studies and the field in general, having reliable antibodies towards a variety of protein targets is critical. Unfortunately, antibodies are expensive and the cheaper polyclonal variety offer inconsistent results.

One way to offset the cost and produce reliable antibodies, is to make monoclonal antibodies in house, which is the focus of my current research. As a first target, I am generating a monoclonal antibody towards the centromeric protein, CeCENP-A^{HCP-3} (HCP-3), in *C. elegans* which is critical for kinetochore deposition during mitosis. More specifically, I am generating a plasmid (pJM20), containing a segment of the N-terminus of HCP-3 in the pGEX-6P-1 backbone. Currently, I have expressed the GST fused HCP-3 N-terminus in BL21(DE3) *E. coli* and confirmed the 36 kD protein product via SDS-PAGE Coomassie Blue staining. Full scale protein production is currently underway, and future directions will focus on protein purification and antibody production. While HCP-3 is only the first target for antibody production, the development of this technology in house will pave the way for generating antibodies against many other proteins, providing opportunities for numerous cell biological studies in the future.

Overall, I have performed a variety of cell biology research during my time at Ramapo. I have learned about many common laboratory practices. These include various live imaging techniques, how to perform gel electrophoresis on both agarose and polyacrylamide gels, how to create a recombinant plasmid vector, how to successfully raise cell cultures, how to induce protein production on a large scale, and various methods of protein purification. In total, I have spent about 500 hours either actively developing or implementing protocols in the laboratory, performing various literature reviews, or preparing my own research summaries and presentations. As I pass this research on to future research students, future goals include mass protein production and protein purification via affinity chromatography. Ultimately, the purified protein will be used to inoculate mice and begin antibody production. Monoclonal HCP-3 antibodies can then be obtained, purified, and cloned. I want to extend a sincere thank you to Dr. Monen, Dr. Owen, Dr. Stuart, and Dr. Kim, as well as to Brianna Romer and Jonathan Lopez, and to members of the Kim Lab: Pritha Aggarwal, Christine Jaipersaud, Soo Jin Koo, and Devashri Parikh. This work was supported by a grant from the Ramapo College Foundation, the TAS Research Honors Program, and the Ramapo College Honors Program.

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Speaking of Research. August 7, 2014; [March 22, 2017]. Antibody Production Illustration provided by Alice Ra'anan and Bill Yates.