Ras Signaling Regulation by Monoubiquitination
Cambrynne Rietberg with Professor Baker

More than one third of all human cancers are caused by small changes in the protein Ras. Located at the top of complex signaling pathways in a cell, Ras works like a light switch that turns the pathways on and off. When Ras is turned on, it regulates many essential processes such as cell growth, differentiation, and apoptosis. However, mutations to Ras cause regulators to be unable to turn Ras off, thus misregulating those essential cell processes and driving cancer. So far, scientists have been unable to target these mutations directly for drug development, leaving room for exploration of alternative contributors to Ras-driven cancers.

Recently, it has been shown that the posttranslational modification of monoubiquitination might also play a role in Ras activation, because when the protein of ubiquitin is bound to specific sites on Ras, the activity of Ras changes. There is also preliminary evidence in mouse models that Ras modification by ubiquitination may be important in cancer. This is exciting because in the future, targeting ubiquitination of Ras has the potential to lead to new approaches to treating Ras-driven cancers.

However, before we can think about how to target this modification in cancer, we first have to understand the role that ubiquitination plays in normal Ras signaling. My approach this summer was to take a molecular biology approach to study Ras ubiquitination using yeast as a model system. As a continuation of my research on Ras signaling from last summer and spring, my goals for the summer were to confirm that Ras is differentially ubiquitinated and to explore the impact of ubiquitination on Ras localization.

Yeast Ras comes in two forms known as isoforms – Ras 1 and Ras 2. First, in order to confirm that Ras is differentially ubiquitinated, I would have to see ubiquitin present in one isoform but not in the other. This study has been performed once before by using a Western blot to visualize ubiquitination in a specific yeast strain that can show enhanced ubiquitination. The results from the replication of this experiment are promising, supporting the previous evidence that Ras 1 is ubiquitinated while Ras 2 is not. I hope to repeat the experiment again with the goal of seeing clearer bands on the Western blot.

I also used a cell fractionation assay to explore if ubiquitination impacts Ras 1 localization. Ras 2 localization has been previously researched, so I am comparing Ras 1 localization to that of Ras 2 and hope to see if localization is ubiquitination-dependent.

Once ubiquitination is clearly visualized in Ras 1, I plan to explore questions of whether the pattern of ubiquitination changes under the presence of various stressors and signals, such as heat stress and glucose signaling, to better understand the conditions under which ubiquitination is used to regulate Ras. In addition to observing changes in the pattern of ubiquitination, I also hope to determine the site of ubiquitination binding, and to continue exploring the impact of ubiquitination on localization. Then, ubiquitination may not only be involved in modulating Ras signaling, but also in cell trafficking. Through all of these experiments, my goal is to gain a better understanding of the role that ubiquitin plays

in yeast Ras signaling so that this could become a target for treatment of Ras-driven cancers.

I am thankful to Calvin College for the opportunity to continue research on this project, to Professor Baker for being a great research mentor, and to the generous donors who also allow this research to be possible. Given my interests in medicine and cancer biology, working on a project that has such clear relevance to health and disease has given me experience that will shape my future. It has been a privilege to learn through the research process, to have to think critically and troubleshoot through each step, and to explore some of the tiniest of life's functions and pathways, what happens when those functions go wrong, and how we can treat these changes in the future.