Developing a new biosensor for Ras activation

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Ras is a protein that becomes mutated in one-third of all human cancers and is involved in cell survival, gene expression, cell cycle progression, and apoptosis. With Ras' involvement in numerous vital functions, it is important to characterize its localization and activation. It is known that Ras is active when guanine tri-phosphate (GTP) is bound and inactive when the di-phosphate version of guanine (GDP) is bound. Ras' function is to turn itself off by hydrolyzing GTP. Enzymes are used to catalyze turning Ras on and off, those enzymes are guanine exchange factor (GEF) and GTPase activating protein, respectively.

Our goal this summer was to use this known information to create a novel system for tracking the localization and activation of Ras in live cells. We did this through a method called biolumiunescense resonance energy transfer (BRET). For this system to work, we needed to connect our protein of interest to a light emitting protein. This light energy will transfer to a fluorophore linked to Ras' activating agent, GTP, so when GTP binds, the fluorophore will excite and emit an energy signature.

To accomplish our goals, we began the summer by creating a DNA construct for Ras and nanoluciferase in a bacterial expression vector. This vector allowed us to have bacterial cells produce our combination protein. Purification of this protein was the next step, and proved to be a challenging one. Since our protein is non-native, cells have a tendency to produce and aggregate it into inclusion bodies. To obtain protein, cells can be stressed to produce a lower quantity as to prevent aggregation, or inclusion bodies can be lysed open. We chose to stress cells because lysing the inclusion bodies would denature our protein, making it not act normally, which is a problem when we want normal functioning Ras. Stressing cells lowered the yield of protein from a purification, but enough was collected to run a few assays that verified its activity.

Finally, with good data acquisition from the purified protein, we were able to move on to the second prong of our study. For this part of the study we have to create our DNA construct in a vector for mammalian cell expression. I have begun this work, but will continue it during the semester to transfect mammalian cells. The novelty behind our method of mammalian cell production is that they will express and secrete our protein into solution for our later studies on Ras activation and localization in live cells.

Our goals were to characterize the activity of Ras-nanoluciferase fusion proteins to verify its similarity to normal Ras, and move that system into live cells for future experimentation to look at Ras activation and localization. Information obtained from our study will provide insight into what function Ras is playing in humans to better understand Ras driven cancers.

I appreciate for the opportunity to explore complex issues through research under the great mentorship available at Calvin College. Research has been a great way to apply, understand, and get involved in science.