Carolyn Eberle

Major: Biochemistry
University: University of Missouri-Columbia
Faculty Mentor: Dr. Mark Hannink
Mentor Department: Biochemistry
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Identifying substrate proteins for GAN1 and Keap1

Carolyn Eberle, Mark Hannink and Zheng Sun

GAN1 and Keap1 are proteins characterized by a N-terminal BTB domain and a C-terminal Kelch repeat domain. Both of these domains are protein-protein interaction domains, suggesting that these BTB-Kelch proteins form signaling complexes in cells. Previous work has suggested that BTB-Kelch proteins function as substrate adaptor proteins for Cul3-dependent E3 ubiquitin ligase complexes. The goal of this project was to identify substrate proteins of GAN1 and Keap1. This information will be particularly useful when for understanding Giant Axonal Neuropathy, a sensorimotor disease characterized by excessive accumulation of neurofilaments in neurons that contain mutated GAN1 genes. We used an affinity purification approach to identify candidate substrate proteins for GAN1 and Keap1. Recombinant GAN1 and Keap1 genes containing a C-terminal chitin binding domain (CBD) were inserted into pBabe puro vectors. These vectors were used to generate virus stocks, which were used to infect a microglial cell line, BV-2. Stable cell lines were generated using puromycin selection. A mock-infected cell line was generated in parallel. When the cell lines were confluent, the cells were lysed using a 0.1% SDS RIPA solution and chitin beads were used to precipitate the CBD-tagged proteins. Western blot analyses were performed to determine if the purification of the CBD-tagged proteins was successful. No CBD-tagged proteins were identified in our first pull-down experiment. We are currently reexamining the precipitation protocol and preparing to lysate the same set of cells.