Usher Syndrome Type IIIB leads to vision and hearing loss, starting in early childhood and progressively worsening throughout the individual's life. A mutation, Y454S, was found in the gene that codes for histidyl-tRNA synthetase (HARS), a critical enzyme for protein translation. Mutant aminoacyl tRNA synthetases are also associated with neural disease, and have been shown to localize differentially in neurons. The aim of this project was to determine if the mutant HARS protein prevented or hindered histidyl-tRNA synthetase from being transported down neural processes as a possible explanation for the mutant phenotype of blindness and deafness. Sensory neural cells (N33) and epithelial cells (E36), derived from mouse embryo ears, were transfected with mutant and wild type HARS as well as the green fluorsescent protetin GFP to visualize the cell body. The cells were fixed, nuclei were stained with DAPI and the transfected histidyl-tRNA synthetase proteins were tagged with fluorescent antibodies. Y454S and wild type histidyl-tRNA synthetase have been imaged in N33 and E36 cell lines. The synthetase appeared to associate with the plasma membrane, thus some E36 cells were also tagged with antibodies for the plasma membrane. Proteomics data suggested Y454S might preferentially locate to proteasomes, so E36 cells were also stained with a proteasome marker. No difference has been found between Y454S and wild type HARS localization in these cells, however the cells were immature and neural processes would not grow. Therefore new work will use the rat neural model PC12 cell, which will grow extended neural processes in vitro. The histidyl-tRNA synthetase and mutant localization will be assessed in the neural processes. This project will continue into next year.