Chad El-Zayaty
(mentor: Gurjit Khurana-Hershey)

STAT (Signal Transducer and Activator of Transcription) proteins are a class of second messengers that activate gene transcription. STAT proteins bind to activated cell surface receptors by their SH2 domains. This allows the STAT protein to be phosphorylated on one specific tyrosine residue. The STAT proteins then dimerize. The homodimer will then translocate to the nucleus and bind to specific DNA sequences. STAT6 is a member of the STAT family of proteins. STAT6 is associated with the cytokine receptor IL-4Rα. It is activated by IL-4 and IL-13, which both utilize IL4Rα as part of their respective receptor complexes. Upon IL-4 binding, associated JAK1 and JAK3 kinases become activated leading to STAT6 activation and translocation to the nucleus. Our central hypothesis is that STAT6 is present in the nucleus at baseline and constantly cycles between the nucleus and the cytoplasm. Our objectives are to show that STAT6 is present in the nucleus at baseline, to show that phosphorylated STAT6 is not present in the cytoplasm, and to show that STAT6 is dephosphorylated in the nucleus. We labeled STAT6 in murine A20 cells with rabbit anti-murine STAT6 followed by a goat anti-rabbit fluorescent antibody. After a short incubation the cells are ready for analysis by either flow cytometry or by confocal microscopy. To track activated or phosphorylated STAT6, we utililized an antibody against phospho-STAT6. This antibody labels the active phosphorylated form of STAT6 exclusively enabling us to test our hypothesis that phosphorylated STAT6 is no longer present in the nucleus. Confocal microscopy has enabled us to track the path of STAT6 at various time periods and see if the fluorescent STAT6 is still present after inactivation. Our experiment demonstrated that this method can be used to label both STAT6 and phosph-STAT6 for observation in a Confocal microscope. We have also demonstrated that phosph-STAT6 can be found in the nucleus, and that STAT6 can be found in both the cytoplasm and the nucleus. Our results are consistent with our hypothesis that STAT6 cycles between the nucleus and the cytoplasm.