Structure, Function, and Inhibition of the Oncogenic RhoGEF TrioC

The C-terminal Guanine Exchange Factor (GEF) domain of the RhoGEF Trio (TrioC) has recently been implicated in highly metastatic cancers including uveal melanoma and adult T-cell leukemia. Upon aberrant activation, TrioC transduces growth and motility signals through the small GTPase RhoA. The regulation of TrioC is not understood on a molecular level and this presents a barrier to future inhibitor design efforts. Preliminary biochemical studies have shown that TrioC is regulated via an autoinhibitory constraint that is released upon the binding of the heterotrimeric G-protein Gαq. I have solved the 2.7Å crystal structure of TrioC in its basal state, revealing high-resolution detail of the autoinhibition mechanism. Analysis of this structure in comparison to the activated state of a closely related protein, p63RhoGEF reveals a dramatically different orientation of key regulatory regions. Mutational analysis of these regulatory regions are underway using FRET-based activity assays and differential scanning fluorimetry. Analysis of current data supports that residues in the α6-αN linker helix and β3-β4 loop regions are indeed responsible for stabilizing the autoinhibited state of TrioC. A Trio inhibitory peptide (TRIPα) represents the only known inhibitor of TrioC. I have expressed the peptide as an MBP fusion and am attempting to determine a co-crystal structure of TRIPα in complex with TrioC. I am also planning to run a high-throughput displacement screen to discover novel chemical matter which binds to TrioC in the same manner as TRIPα.