The Ras small GTPases function as binary switches to transduce mitogenic stimuli from the extracellular environment to the nucleus. The first Ras effectors to be identified were the Raf serine/theronine kinases (Chong *et al.*, 2003; Wellbrock *et al.*, 2004). Activated Raf phosphorylates and activates the MEK1 and MEK2 dual specificity protein kinases, which then phosphorylate and activate ERK serine/threonine kinases. Activated ERK subsequently phosphorylates many substrates resulting in changes in gene expression that regulate cell cycle progression and survival. The importance of Ras activation in oncogenesis is supported by the identification of activating point mutations in over 30% of human cancers (Malumbres *et al.*, 2003). Mutational activation of Raf is also frequently identified, *e.g.* in 70% of melanomas and 10% of colorectal carcinomas (Wellbrock, Karasarides *et al.*, 2004). The non-overlapping occurrence of Ras and Raf mutations suggest that activation at either level is sufficient for cellular transformation and that the Ras-Raf-MEK-ERK pathway is of primary importance to tumor formation.

The high prevalence of activating mutations in Ras and Raf in cancer has led to considerable effort in targeting these proteins with small molecules as a novel cancer therapeutic approach; however the complexity and redundancy of the Ras pathways have made this an arduous task. While the Ras-Raf-MEK-ERK cascade is frequently depicted as a linear pathway from the plasma membrane to the nucleus, scaffolding proteins such as Sef, MP1, and KSR are crucial to the spatial and temporal aspects of activation by simultaneously binding multiple components of the signaling cascade. Work in the Siderovski lab has demonstrated that a novel scaffold protein, RGS12, binds TrkA, activated H-Ras, B-Raf and MEK2, facilitating prolonged ERK activation (Willard *et al.*, 2007) and that RGS12 is expressed in colorectal and melanoma cell lines, suggesting a role in coordinating signaling from oncogenic alleles of Ras and Raf (unpublished data). While the architecture of RGS12 and RGS14 is similar (Figure 1) it is currently unknown if RGS14 can affect Ras-Raf-MEK-ERK signaling in a similar way to RGS12. The ability of RGS12 and potential ability of RGS14 to assemble multiple components frequently associated with tumorigenesis suggest that small molecules that target specific interactions between these scaffolds and their substrates will provide useful tools to interrogate the role of RGS12 and RGS14 in oncogenic transformation and provide a novel therapeutic approach toward cancer. My research goals were two fold:

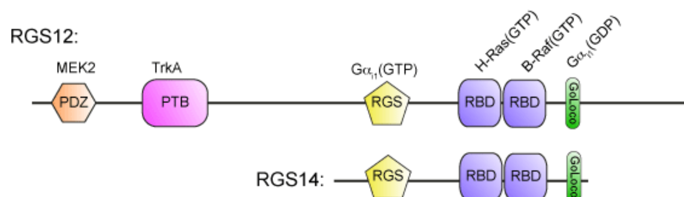

Figure 2. RGS12 and RGS14 bind multiple components of the Ras-Raf-MEK-ERK pathway. Based on this multiple domain arthitecture previous studies in our lab have demonstrated the ability of RGS12 and RGS14 to bind to MEK2, TrkA, Gα(GTP/GDP), activated H-Ras and activated B-Raf.

1. Determine if RGS14 can assemble a complex of Ras, Raf, MEK, and ERK to modulate p-ERK signaling
2. Identify small molecules that will target the specific domains within RGS12 and RGS14 as a novel therapeutic approach to cancer therapeutics

Toward the first aim of my dissertation proposal, we recently published a paper in which we found that RGS14 selectively binds to mutationally activated H-Ras *in vivo* and not to related Rap family GTPases (Willard *et al.*, 2009). Although RGS12 and RGS14 have similar domain structure, we were unable to see direct binding of RGS14 to B-Raf without the presence of mutationally activated H-Ras, unlike RGS12. Knockdown of RGS14 levels inhibited neuronal growth factor (NGF)- and basic fibroblast growth factor (bFGF)-mediated p-ERK accumulation and cellular phenotypic changes associated with p-ERK signaling. These results indicate that RGS14 is an atypical Ras-Raf-MEK-ERK signaling scaffold and suggest that targeting such a component of the canonical Ras > p-ERK signaling pathway with small molecules could have therapeutic effects.

Toward the second aim of my dissertation proposal, I published a first-author paper describing the precise molecular mechanism by which CCG-4986, a putative RGS protein inhibitor, exerts its effects (Kimple *et al.*, 2007). This work revealed the reactive nature of CCG-4986 and identified the particular surface-exposed cysteine on the RGS domain that is targeted by the compound to affect substrate docking. In addition, to

identify new chemical matter that will inhibit the RGS domain of RGS12 and RGS14, I developed the first steady-state GTPase assay for this class of proteins that can be automated and run in high-throughput (HTS) format (Kimple *et al.*, 2009). This co-first author publication is an exciting advance towards identifying RGS small molecule inhibitors as it allows the measurement of enzymatic activity instead of RGS/substrate binding which is a poor surrogate for enzymatic activity. In addition to my efforts in identifying small molecules to target the RGS domain in RGS12 and RGS14, I have also worked with the Structural Genomics Consortium (Oxford, U.K.) to help solve X-ray and NMR structures of all human RGS proteins in order to establish the structural determinants underlying their specificity towards particular binding partners. This work resulted in 14 new RGS protein structures (NMR and X-ray) that have allowed us to gain critical insight into the molecular determinants of RGS selectivity toward binding partners (Soundararajan *et al.*, 2008), as well as a follow-up first-author paper specifically on the unique specificity of RGS2 (Kimple *et al.*, 2009). This detailed understanding of how different RGS proteins engage binding partners will be valuable for compound library drug screening as well as rational and in silico drug design.

In an additional first-author publication, I collaborated with the North Carolina Central University's Biomanufacturing Research Institute and Technology Enterprise (BRITE) and the NIH Chemical Genomics Center (NCGC) to develop an HTS assay to screen for small molecules that inhibit the binding of RGS12 to inactive $G\alpha_{i1}$ (Kimple *et al.*, 2008). Based on mutagenesis studies (Sambi *et al.*, 2006), disruption of this interaction should result in mislocalization of RGS12 to the nucleus and thereby inhibit its ability to act as a scaffold for Ras-Raf-MEK-ERK (Willard, Willard *et al.*, 2007). My paper described the development of a novel fluorescence polarization assay, its miniaturization from 96-well format to 384-well format and then 1536-well format, as well as an initial screen of ~30,000 compounds. We are currently validating hit compounds in follow-up cellular assays, as well as using medicinal chemistry expertise here on campus to optimize leads for in vivo efficacy. In parallel to these studies, I worked with a post-doc in our lab to identify and extensively validate point mutants of $G\alpha$(GDP) that abolish binding to RGS12 and RGS14 *in vitro* and *in vivo* but do not affect any other aspect of $G\alpha$ signaling (Willard *et al.*, 2008). These efforts results in a co-authored publication that will facilitate future studies into how the RGS12/14-$G\alpha$i1(GDP) interaction affects normal cell division and oncogenesis.

The pharmaceutical industry is rapidly transforming as it becomes clearer that their current research and development models will not sustain blockbuster pipelines. My basic science background in elucidating signal transduction pathways, coupled with my experience in assay development and chemical biology, and my continuing medical/clinical training, will allow me to become well-poised for a career in academic medicine in translational oncology drug discovery.

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