RAF restrained and ready for RAS

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RAF family kinases lie at the top of the three-tiered MAP kinase cascade, and are directly activated by RAS in response to growth factor stimulation. RAF activity must be tightly controlled, as inappropriate activation leads to cancer. In particular, the oncogenic BRAF V600E mutant drives more than 50% of malignant melanoma and is also found in many other cancers. The conserved domain structure of RAF proteins includes an N-terminal regulatory region as well as the C-terminal kinase domain. The N-terminal region consists of a RAS-binding domain (RBD) and a cysteine-rich domain (CRD) that mediates membrane association. RAF activity is thought to be controlled by autoinhibitory interactions of the N-terminal region with the kinase domain, and by a complex set of phosphorylation sites. Two of these sites flank the kinase domain and mediate association with 14-3-3 proteins, while others lie in the kinase and activation segment. Despite decades of study, we lack a detailed mechanistic understanding of RAF regulation; crystal and/or NMR structures have only been available for isolated domains of RAF family members, precluding an integrated understanding of its autoregulation. We are working to understand BRAF regulation and have succeeded in preparing the intact kinase in an autoinhibited form for structural and mechanistic studies. We will describe our progress in using cryo electron microscopy to elucidate the structure of autoinhibited BRAF and its interactions with 14-3-3 proteins and its substrate MEK.