

## **Regulation of Btk by the SH2-kinase Linker Region**

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Src, Tec, Abl and ZAP70 family kinases are non-receptor tyrosine kinases that play critical signaling roles in cell. All these kinases contain an SH2-linker-kinase domain structure, indicating a biological significance of this arrangement during evolution. Crystal structure and mutagenesis studies from Src family kinases suggest the linker region plays important roles in the inter-domain regulation of these kinases.

Mutations in Bruton's tyrosine kinase (Btk), a Tec family kinase, cause X-linked agammaglobulinemia (XLA) in humans and X-linked immunodeficiency (xid) in mice due to defective B cell development and function. Some XLA-causing mutations are found in the SH2-kinase linker region. Because of the well-established genetic and biochemical tools for Btk, we decided to use Btk/XLA/xid as a model system to study the biological function of this linker region.

Point mutations were introduced into the conserved residues in the Btk linker region. These mutants were then expressed in Btk-deficient DT-40 B cells and the signaling pathway upon B cell receptor stimulation was studied. Two mutants identified from XLA patients (Btk L369F and Btk R372G) showed a paradoxical phenotype: they retained kinase activity, but lost their ability to mediate calcium response and phospholipase C activation in B cells. Two putative tyrosine phosphorylation sites in the linker region are also currently studied. The understanding of the intramolecular regulation of these kinases may enable the identification of less conserved motifs outside of the kinase domain as targets for novel therapeutic kinase inhibitors.