## Phylogeny

Tyrosine-protein kinase ZAP-70 is a member of the Syk family within the tyrosine kinase group of the human kinome and is evolutionarily related to Syk, its B-cell homologue (Deindl et al., 2007; Béné, 2006; Chen et al., 2020; Yan et al., 2013). Orthologues are conserved from fish to mammals, including mouse and rat (Shah et al., 2016; Fischer et al., 2010; Fernández-Aguilar et al., 2023).

## Reaction Catalyzed

ATP + protein-L-tyrosine ⇌ ADP + protein-L-tyrosine-phosphate (Huber et al., 2015; Yan et al., 2013).

## Cofactor Requirements

Requires divalent cations Mg²⁺ or Mn²⁺ for catalytic activity (Béné, 2006; Chen et al., 2020; Deindl et al., 2007).

## Substrate Specificity

The kinase domain recognizes tyrosine sites embedded in sequences of high local negative charge, favouring acidic residues (Asp/Glu) and excluding basic residues (Lys/Arg); this is mediated by a positively charged binding surface on ZAP-70 (Shah et al., 2016). Peptide-array studies report differing consensus motifs:  
• strong P-1 acidic preference and polar residues at P-3/P-2, with small non-polar residues downstream (Regulation of protein tyrosine kinase ZAP-70 by serine phosphorylation, 2003);  
• enrichment of acidic residues at P-3/P-2 and Ile/Val at P + 1 (Yaron-Barir et al., 2024);  
• an alternative view of aliphatic hydrophobic residues at P-1 and P + 3 (Yaron-Barir et al., 2024).

The SH2 tandem domains recognise doubly phosphorylated ITAMs with the consensus (D/E)xxYxxL(x)₆–₈YxxL (Béné, 2006; Deindl et al., 2007).

## Structure

ZAP-70 (~70 kDa) contains two N-terminal SH2 domains and a C-terminal kinase (SH1) domain (Fischer et al., 2010; Béné, 2006). The kinase adopts a bilobal fold: an N-lobe of five β-strands plus the αC helix that anchors ATP, and a C-lobe housing the substrate-binding site (Huber et al., 2015). Key motifs include HRDLAARN and an activation loop between DFG and APE sequences; K369–E386 salt-bridge stabilises the inactive αC helix (Huber et al., 2015). The autoinhibited structure is captured in PDB 2OZO (Deindl et al., 2007).

## Regulation

Basally autoinhibited, ZAP-70 is recruited to T-cell receptor ITAMs phosphorylated by Lck; SH2 binding relieves inhibition (Deindl et al., 2007; Chen et al., 2020). Full activation requires Lck-mediated phosphorylation of:  
• Y492/Y493 in the activation loop (Yan et al., 2013; Fischer et al., 2010);  
• Y315/Y319 in the SH2-kinase linker (Deindl et al., 2007; Williams et al., 1999);  
• Y292, generating a c-Cbl docking site for negative regulation (Kong et al., 1996; Targeting ZAP-70 protein kinase in T-cell lymphoproliferative malignancies, 2024).

## Function

Highly expressed in thymocytes, peripheral T cells and NK cells, ZAP-70 is essential for adaptive immunity (Fischer et al., 2010; Fernández-Aguilar et al., 2023). Downstream of Lck, it phosphorylates adaptor proteins LAT and LCP2/SLP-76, assembling signaling complexes that drive Ca²⁺ mobilisation, MAPK activation, cytoskeletal reorganisation and IL-2 production, thereby regulating thymocyte development, T-cell motility and activation (Béné, 2006; Deindl et al., 2007; Chen et al., 2020).

## Inhibitors

ATP-competitive inhibitors include staurosporine (Huber et al., 2015; Deindl et al., 2007). Additional experimental agents are CPI-818 and cerdulatinib, and fragment screening has revealed an allosteric pocket adjacent to the activation loop (Targeting ZAP-70 protein kinase in T-cell lymphoproliferative malignancies, 2024; Huber et al., 2015).

## Other Comments

Loss-of-function mutations in ZAP70 cause autosomal-recessive severe combined immunodeficiency with absent CD8⁺ and dysfunctional CD4⁺ T cells; hypomorphic variants can predispose to autoimmunity (Béné, 2006; Fischer et al., 2010). Aberrant ZAP-70 expression in B-cell chronic lymphocytic leukaemia is a poor-prognostic marker linked to heightened antigen-receptor signalling (Béné, 2006; Targeting ZAP-70 protein kinase in T-cell lymphoproliferative malignancies, 2024).

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