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Proximal tyrosine kinases that initiate T cell activation

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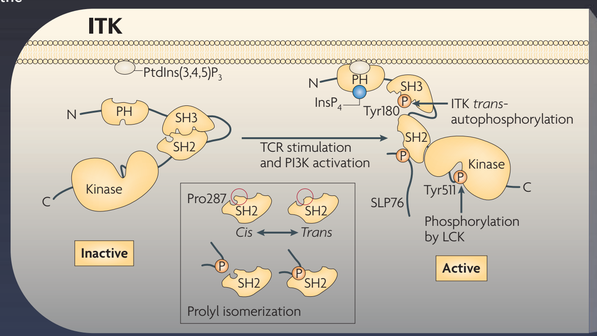
T cells are central to a successful immune response. The proximal tyrosine kinases that convert TCR engagement into a complex series of enzymatic events are crucial for T cell activation, which culminates in the generation of T cell effector functions. Through the characterization of rare human diseases and genetically engineered animal models, in combination with high-resolution structural data, a clearer picture is emerging as to how these proximal tyrosine kinases are regulated by structurally unique mechanisms. A few, but not a comprehensive review, of the well-understood regulatory mechanisms are depicted in this poster. This information has also helped to elucidate how small molecule inhibitors could mechanistically block proximal tyrosine kinase activity, which is essential to improve rational drug design, and supports the idea that proximal tyrosine kinase inhibitors can function as therapeutic drugs to modulate the T cell-mediated immune response.

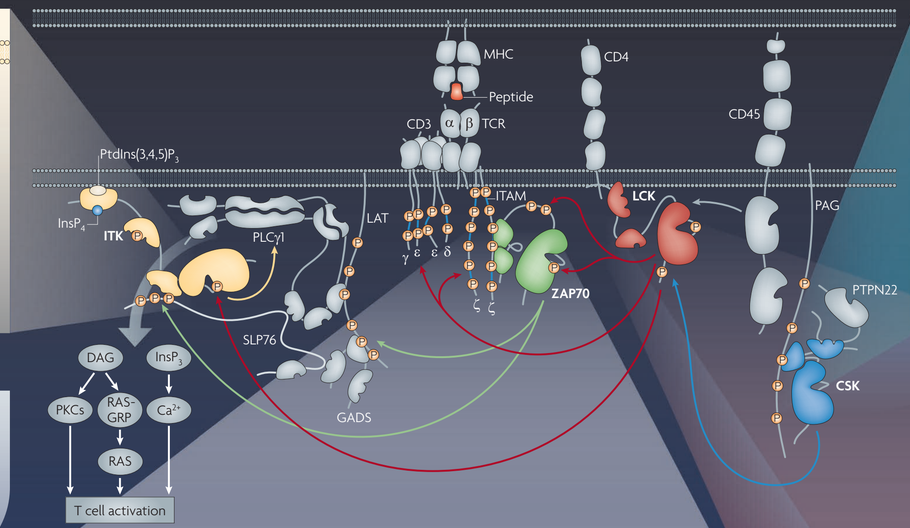
ITK

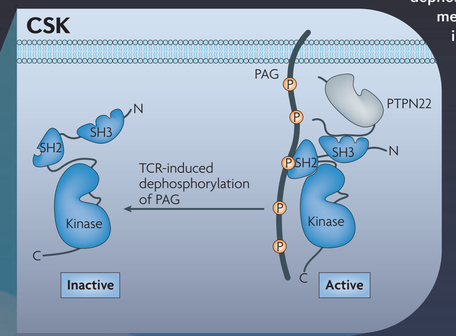
ITK is held in an inactive conformation by an SH2–SH3 domain interaction. Following TCR stimulation, the PH domain of ITK binds PtdIns(3,4,5)P_3, the result of the activation of PI3K, inducing plasma membrane localization of ITK. InsP_4, generated by the action of InsP_3 kinase B, augments this PtdIns(3,4,5)P_3 interaction. Once at the membrane, ITK is activated by LCK-mediated phosphorylation on Tyr511. This is then followed by *trans*-autophosphorylation on Tyr180, which induces the opening of ITK and brings the SH2 domain into contact with the kinase domain, allowing the SH2 domain to bind phosphorylated SLP76. ITK is also regulated by prolyl isomerization at Pro287 in the SH2 domain: the *trans* conformation has a greater affinity for phosphorylated tyrosine residues compared with the *cis* conformation.

TCR signalling

The TCR is a multi-subunit complex that initiates an intricate series of signalling events. In a resting state, the SRC family kinase LCK is usually associated with the co-receptor CD4 or CD8. Following engagement of the TCR and a co-receptor by a peptide-bound MHC molecule, LCK phosphorylates ITAMs present in the cytoplasmic regions of the CD3 chains and TCR ζ-chains. ZAP70 then binds to doubly phosphorylated ITAMs resulting in a conformational change that allows for its phosphorylation by LCK. Phosphorylation of ZAP70 promotes its stabilization in an active conformation. Activated ZAP70 phosphorylates the adaptor molecules LAT and SLP76, which have a central role in nucleating the recruitment of downstream effector molecules. During this process, a third protein tyrosine kinase, ITK, is recruited to a LAT-containing multimolecular signalling complex at the plasma membrane. ITK phosphorylates and activates PLCγ1, inducing the production of the crucial second messengers InsP_3 and DAG, which lead to an increase in cytoplasmic Ca^{2+} concentration and to the activation of RAS and PKCs, respectively. These events activate further downstream signalling pathways leading to T cell activation.



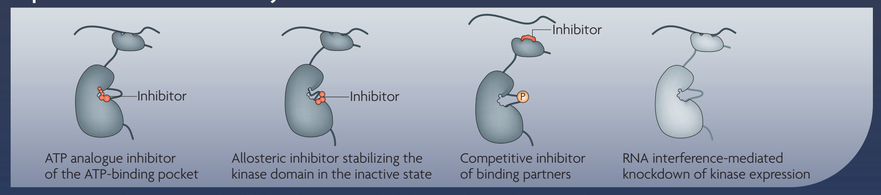




CSK

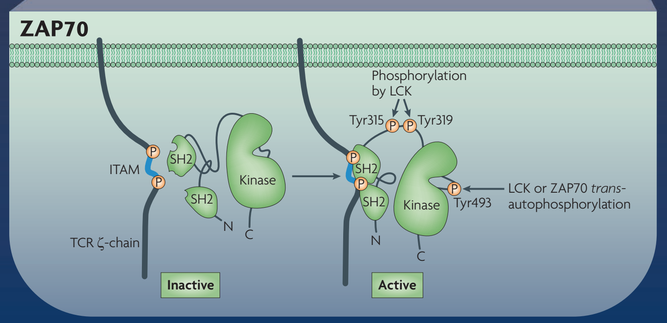
When anchored to the plasma membrane by association with PAG, CSK is active owing to the conformation of both linker regions with respect to the kinase domain and an SH2–kinase domain interaction. TCR stimulation induces dephosphorylation of PAG through unknown mechanisms, resulting in the release and inactivation of CSK. Importantly, PAG-deficient mice have no appreciable defect in CSK function indicating that functionally redundant molecules are likely to exist. Interestingly, the PTPN22 (also known as LYP) cytoplasmic phosphatase contains a proline-rich sequence that interacts with the SH3 domain of CSK. PTPN22 dephosphorylates the activation loop tyrosine of SRC family kinases. Thus, CSK and PTPN22 act in tandem to negatively regulate SRC family kinases. The interaction of PTPN22 with CSK is disrupted in the allele of the phosphatase associated with autoimmunity.

Experimental inhibitors of tyrosine kinases



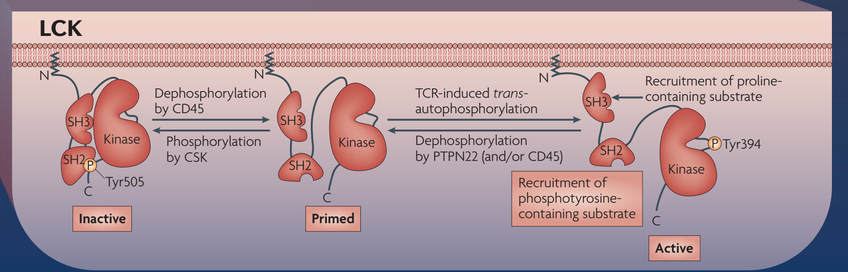
Most current inhibitors target the ATP-binding region of the kinase domain; however this strategy poses many limitations owing to the conserved catalytic mechanism of all tyrosine kinases. In light of more recent structural data, allosteric inhibitors are now being designed to stabilize the inactive conformation of the kinase. Also, some inhibitors are being designed to inhibit the recruitment of the kinase to its proper location by blocking its association with binding partners. More recently, RNA interference-mediated knockdown of kinase expression has shown some promise, although this approach is still in its infancy.

Tyrosine kinase	Human mutation	Mouse knockout
LCK	• $CD4^+$ T cell lymphopenia • Diminished *in vitro* response to mitogens • Generally preserved early TCR signalling	• Decreased number of DP and SP thymocytes, as well as peripheral T cells • Decreased TCR signalling and responses • Complete block at DN thymocyte stage when combined with *Fyn* knockout
ZAP70	• Absence of peripheral $CD8^+$ T cells • $CD4^+$ T cells present but have a TCR signalling defect (compensation by SYK, expressed by thymocytes but not peripheral $CD4^+$ T cells)	• Arrest at DP to SP thymocyte stage affecting both $CD4^+$ and $CD8^+$ T cells • Complete block at DN thymocyte stage when combined with *Syk* knockout
ITK	• Immunodeficiency syndrome • Fatal inadequate response to EBV • Similar phenotype to XLP caused by defects in SAP or XIAP	• Mild immunodeficiency owing to redundancy with other TEC family kinases • Population of non-conventional, innate-like $CD8^+$ T cells
CSK	• No known human mutations • PTPN22 polymorphism that decreases association with CSK predisposes to autoimmunity	• Early embryonic lethal phenotype • Depletion in early immature thymocytes bypasses need for pre-TCR and TCR signalling as a result of increased basal LCK and FYN activity



ZAP70

Binding of the tandem SH2 domains of ZAP70 to a doubly phosphorylated ITAM (Yxx(L/I)x_{7-8}Yxx(L/I)) in the cytoplasmic regions of the CD3 chains or TCR ζ-chains induces a conformational change in ZAP70 that increases activity of the kinase and results in accessibility of Tyr315 and Tyr319 for phosphorylation by LCK. Activation of ZAP70 is further potentiated by Tyr493 phosphorylation by LCK or by ZAP70 *trans*-autophosphorylation.



LCK

When LCK is phosphorylated on Tyr505, intramolecular interactions (between the SH2 domain and phosphorylated Tyr505 and between the SH3 domain and interdomain) hold LCK in a closed, inactive conformation. Dephosphorylation of Tyr505 by CD45 'unlocks' LCK into a primed state. *Trans*-autophosphorylation of Tyr394, which can result from clustering of co-receptors or decreased CSK function, results in full activation of LCK. LCK is inactivated by dephosphorylation of Tyr394 by the phosphatase PTPN22 (and/or CD45) and phosphorylation of Tyr505 by CSK. The phosphorylation status of LCK is probably a dynamically controlled process subject to relocalization of CD45 and/or CSK from their substrate LCK. Note that in some cases the related SRC family kinase FYN can substitute for LCK function or may be activated by LCK to promote downstream substrate phosphorylation.

Epinova DPU

In its quest to find new innovative medicines GlaxoSmithKline (GSK) has embraced the concept of forming specialised science-focussed discovery performance units (DPUs). EpiNova-DPU was formed by the Immune-Inflammation Centre of Excellence for Drug Discovery (II-CEDD) along entrepreneurial biotech lines in January 2009. This was to cluster GSK's ambition in prosecuting epigenetic targets with its established drug discovery expertise in pathway control. EpiNova's remit is to identify small molecule drugs to regulate key molecular switches by focussing internal resource on epigenetic modulators, and by working with key technology-driven partner companies to increase the possibility of success of developing new drugs to regulate signalling pathways, such as with Cellzome and kinases. www.gsk.co.uk

Cellzome

Cellzome is a drug discovery and development company using its chemical proteomics technologies to identify novel drug candidates for the treatment of inflammatory diseases. Driven by *Kinobeads*™, a proprietary technology for screening and profiling of kinases in their physiologically-relevant context, Cellzome has developed a pipeline of innovative inhibitors of key inflammatory kinases, including several which act downstream of the TCR. Analogous to *Kinobeads*™, a new chemical proteomics technology has been developed termed *Epispher*e™, which addresses epigenetic target classes, with the goal of developing novel drug candidates targeting epigenetic enzymes in their disease relevant complexes. The company has also pioneered the large scale application of *Pathway Mapping* for the elucidation of protein-protein interaction networks underlying key disease pathways. Cellzome is applying its technologies to develop its proprietary pipeline and in its drug discovery collaborations with top-tier pharma, such as GSK. Cellzome has laboratories in Heidelberg, Germany and Cambridge, UK. www.cellzome.com

Abbreviations

CSK, carboxy-terminal SRC kinase; DAG, diacylglycerol; DN, double negative; DP, double positive; EBV, Epstein–Barr virus; GADS, GRB2-related adaptor protein; GRP, guanosine releasing protein; InsP_3, inositol-1,4,5-trisphosphate; InsP_4, inositol-1,3,4,5-tetrakisphosphate; ITAM, immunoreceptor tyrosine-based activation motif; ITK, interleukin-2-inducible T cell kinase; LAT, linker for the activation of T cells; LCK, lymphocyte-specific protein tyrosine kinase; PAG, phosphoprotein associated with glycosphingolipid microdomains; PH, pleckstrin homology; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLCγ1, phospholipase Cγ1; Pro, proline; PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-trisphosphate; PTPN22, protein tyrosine phosphatase non-receptor type 22; SAP, SLAM-associated protein; SH, SRC homology; SLP76, SH2-domain-containing leukocyte protein of 76 kDa; SP, single positive; TCR, T cell receptor; Tyr, tyrosine; XIAP, X-linked inhibitor of apoptosis; XLP, X-linked lymphoproliferative syndrome; ZAP70, ζ-chain-associated protein of 70 kDa.

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Competing financial interests

The authors declare competing financial interests: see Web version for details.

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