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Review

# The Src, Syk, and Tec family kinases: Distinct types of molecular switches

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ABSTRACT

The Src, Syk, and Tec family kinases are three of the most well characterized tyrosine kinase families found in the human genome. Members of these kinase families function downstream of antigen and $F_c$ receptors in hematopoietic cells and transduce signals leading to calcium mobilization, altered gene expression, cytokine production, and cell proliferation. Over the last several years, structural and biochemical studies have begun to uncover the molecular mechanisms regulating activation of these kinases. It appears that each kinase family functions as a distinct type of molecular switch. This review discusses the activation of the Src, Syk, and Tec kinases from the perspective of structure, phosphorylation, allosteric regulation, and kinetics. The multiple factors that regulate the Src, Syk, and Tec families illustrate the important role played by each of these kinases in immune cell signaling.



**Contents**



## 1. Introduction

Protein kinases demonstrate a wide variety of activation mechanisms. Factors known to mediate activation include phosphorylation, binding of allosteric regulators, and changes in cellular localization [1,2]. Despite this diversity of regulation, until recently few kinases had been characterized used enzymology and biophysical methods to better understand the molecular basis of activation. Fortunately, there recently has been an increase in the number of structural and quantitative investigations probing the molecular basis of kinase activation, particularly for members of the Syk and Tec family of tyrosine kinases.

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There has also recently been a realization that kinases often function at nodes in signaling networks and can act as molecular switches that control signaling flux through a network [3–6]. How a kinase regulates signaling through a node is determined by the type of "kinase switch" found at that node, and several different types of kinase switches have been described (See Section 5). Determining how multiple factors (phosphorylation, allosteric ligands, etc.) cooperate to cause activation helps characterize a kinase as a particular switch and hence provides a better understanding of the role of the kinase within the signaling network.

This review focuses on the Src, Syk, and Tec family of kinases. These kinases signal immediately downstream of antigen and $F_c$ receptors in immune cells [7–10] (Fig. 1). In Section 2 of this review, the particular tyrosine kinases found immediately downstream of antigen receptors (Lck, Fyn, Zap-70, and Itk in T cells; Lyn, Syk, and Btk in B cells) are introduced; this section focuses on the biological role of each kinase in immune cell signaling. In Sections 3 and 4 of the review, biochemical studies characterizing activation of the Syk and Tec family kinases are described. In Section 5, mechanistic similarities and differences between the Src, Syk, and Tec family of kinases are discussed, and each family is proposed to be a distinct molecular switch based on its unique activation mechanism.

## 2. Src, Syk, and Tec family kinases in lymphocyte signaling

There are 90 tyrosine kinases among the 518 kinases in the human genome [11]. Of these, 32 are nonreceptor tyrosine kinases and 58 are receptor tyrosine kinases. The nonreceptor tyrosine kinases contain 10 subfamilies referred to as ABL, ACK, CSK, FAK, FES, FRK, JAK, SRC, TEC, and SYK [12]. The Src family is the largest of these subfamilies comprising 8 kinases (Fgr, Fyn, Src, Yes, Blk, Hck, Lck, Lyn). The Tec family has 5 kinases (Bmx, Btk, Itk, Tec, Txk) while the Syk family has 2 kinases (Syk, Zap-70).

Members of the Src, Syk, and Tec family of kinases comprise a conserved signaling unit that operates downstream of many different receptors. Examples of the signaling unit found in T and B cells are shown in Fig. 1 [7,8]; similar signaling events also occur in mast cells, macrophages, and in some non-immune cell types [9,10]. Within this

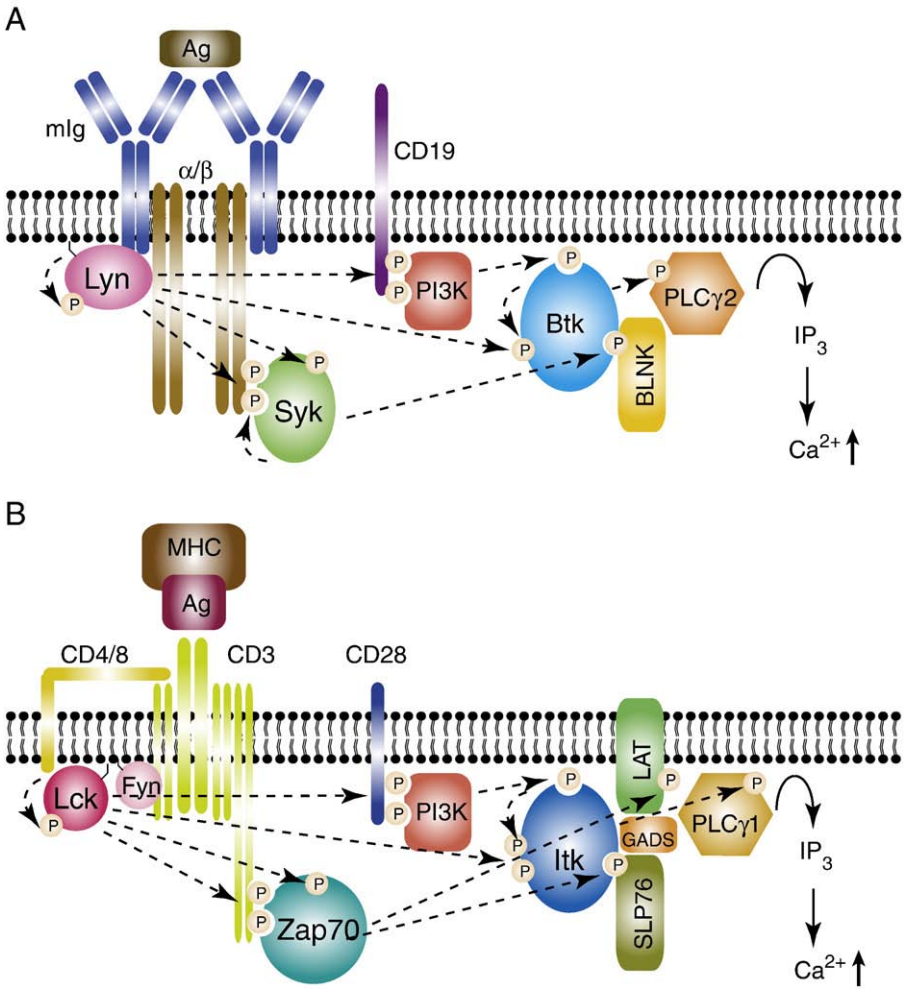


**Fig. 1.** Role of Src, Syk and Tec kinases in B and T lymphocytes. A) Kinase signaling downstream of the B cell receptor (BCR) [8]. Binding of antigen (Ag) to the BCR increases activity of the associated Src family kinase Lyn. Lyn phosphorylates itself, furthering increasing its activity, and also other intracellular tyrosines including those within the ITAM motif of the BCR. The phosphorylated ITAM binds and activates Syk. Lyn also phosphorylates CD19 which induces PI 3′-kinase to synthesize phosphatidylinositol 3,4,5 triphosphate (PtdIns (3,4,5)$P_3$) in the plasma membrane. PtdIns (3,4,5)$P_3$ recruits the Tec kinase Btk to the plasma membrane via its PH domain. Btk becomes activated via both Lyn phosphorylation and autophosphorylation. Btk is the major kinase which phosphorylates phospholipase Cγ2 (PLCγ2), resulting in increased inositol triphosphate ($IP_3$) and eventually a spike in intracellular calcium concentration. B) Kinase signaling downstream of the T cell receptor (TCR) [7]. Presentation of the antigen (Ag)/major histocompatibility complex (MHC) to the TCR increases activity of the associated Src family kinases Lck, and also Fyn. Lck phosphorylates several nearby tyrosine residues including those within the ITAM motif of the TCR. The phosphorylated TCR ITAM binds and activates Zap-70. Lck activation also leads to recruitment of the Tec family kinase Itk through activation of PI 3′-kinase. Itk binds to a signaling complex containing LAT, SLP76, and GADS and is involved in activation of phospholipase Cγ1 (PLCγ1). PLCγ1 activation increases the level of intracellular $IP_3$ and $Ca^{2+}$, leading to further downstream signaling events.

signaling unit, each particular kinase family member plays a similar role independent of cell type.

### 2.1. Src family kinases

Src family kinases initiate intracellular signaling in response to ligation of antigen receptors [13,14]. These kinases, which associate with the plasma membrane and often physically link to receptors, become activated via a poorly understood mechanism that involves clustering of receptors. Lyn is the primary Src family member involved in signaling downstream of the B cell receptor [13], while Lck and Fyn are both involved in signaling downstream of the T cell receptor [14] (Fig. 1). Lyn plays an unusual, 2-fold role in B cell receptor signaling; it is essential for initiation of signaling but is also later involved in negative regulation of the signal [15]. The latter point is illustrated by the fact that *lyn*$^{-/-}$ B cells are hyper-responsive to B cell receptor ligation [16,17]. It is now clear that Lyn activates both positive and negative regulatory proteins in B cell receptor signaling [18]. In T cells, Lck and Fyn also appear to play a dual positive and negative role in signaling downstream of the T cell receptor [19]. *Lck*$^{-/-}$ mice have defects in thymocyte maturation not seen in *Fyn*$^{-/-}$ mice [20–22], potentially indicating a stronger requirement for Lck in T cell development.

Src family kinases were the first nonreceptor tyrosine kinases to be well characterized with regard to their structure and biochemical mechanism of activation [23–25] and will only be briefly discussed in this review. Src kinases are defined by their conserved domain structure consisting of an N-terminal membrane localization motif, a unique region, a Src homology 3 (SH3) domain, a Src homology 2 (SH2) domain, a kinase domain, and a C-terminal, regulatory tail (Fig. 2). The crystal structure of the Src and Hck kinases determined the relationship between these domains for inactive Src family kinases [26,27]. In these structures, a phosphorylated tyrosine (Tyr 527 in Src) within the tail is bound to the SH2 domain through an intra-molecular interaction. Furthermore, a segment of the SH2 domain-kinase linker binds the SH3 domain and packs against the back of the kinase domain. Together, these interactions appear to distort the kinase domain preventing full activity.

Biochemical and enzymatic characterizations of Src family kinases supported the notion that both the SH2-tail and SH3-linker interactions are important for down-regulation of kinase activity [24,28,29]. For instance, a version of Src lacking the C-terminal tail is constitutively active and dephosphorylation of Tyr 527 within the C-terminal tail activates the enzyme [28]. Furthermore, protein–protein interactions that destabilize the inactive conformation also activate Src family kinases; for instance, binding of the SH3 domain-containing protein Nef to the SH2-kinase linker enhances Src family kinase activity [30]. In addition to these mechanisms, phosphorylation of the activation loop tyrosine (Tyr 416 in Src) also increases Src family kinase activity [31]. Hence, biochemical and enzymatic characterizations indicate that Src family kinases have multiple mechanisms to increase enzymatic activity.

### 2.2. Syk family kinases

Syk family kinases are activated downstream of Src kinases. The Syk activation process is initiated when Src kinases phosphorylate conserved sequences within receptors known as immune tyrosine activation motifs (ITAMs) [32]. These motifs (containing the sequence: YxxI/Lx$_{7-12}$YxxL) are bound by Syk kinases via interaction with the tandem SH2 domains of the kinase. Binding of Syk kinases to ITAM sequences serves both to activate kinase activity and localize the kinase near its substrates.

Syk and Zap-70 are the only 2 members of the Syk family of kinases. They contain a domain structure consisting of 2 N-terminal SH2 domains separated by an inter-SH2 domain linker (interdomain A), a kinase-SH2 domain linker (interdomain B), and a C-terminal kinase domain (Fig. 2) [33]. Syk and Zap-70 share 56% overall sequence identify with 60% identify within the kinase domain, 65% identify within the inter-SH2 domain linker, and about 55% similarity within the tandem SH2 domains [34]. The kinase-SH2 domain linker shows only 30% sequence identity between kinases and is also the site of a 23 amino acid insert found in Syk but not Zap-70. Interestingly, an alternative splice variant of Syk known as SykB also lacks this 23 amino acid insert [35,36]. SykB has been shown to regulate the ability of Syk to bind ITAM sequences and was found to be less efficient than Syk in coupling receptor activation to downstream signaling events [37].

#### 2.2.1. Syk

Syk (Spleen tyrosine kinase) was first identified through isolation of tyrosine kinase activity found in both thymus and spleen leading to the identification and cloning of the 72 kDa Syk protein [38,39]. Syk was subsequently established to play a key role in signaling downstream of the B cell receptor [40,41]. Analysis of Syk deficient mice revealed that syk$^{-/-}$ lymphoid cells were deficient in B cell maturation and expansion [42]. (Interestingly, Syk knockout mice die perinatally due to severe hemorrhaging, also suggesting a role for Syk in maintaining vascular integrity.) Syk has subsequently been found to play a broad role in haematopoietic cell signaling including downstream of the in FCεRI receptor in mast cells, FCγRIIA receptor in macrophages, monocytes, and platelets, and sometimes the T cell receptor in T cells [43]. In all cases, it appears that receptor stimulation results in recruitment of Syk to ITAM sequences although the sequence and spacing of ITAMs vary between different receptors. While most studies have focused on hematopoetic cells, Syk has also been shown to be expressed in nonhematopoetic cells including fibroblasts, epithelial cells, hepatocytes, neuronal cells, and vascular endothelial cells [44]. Of note, Syk has been reported to be a negative regulator of tumorigenesis in breast cancer cells [45]. The central role of Syk in multiple lymphocyte signaling pathways has focused attention on Syk as a potential target for treatment of inflammatory diseases such as rheumatoid arthritis, asthma, and allergic rhinitis [46,47].

#### 2.2.2. Zap-70

Zap-70 (Zeta-chain associated kinase of 70 kDa) was first identified as a protein associated with the ζ chain of the T cell receptor [48]. Determination of the amino acid sequence revealed that Zap-70 was a tyrosine kinase with homology to Syk [49]. Unlike Syk, Zap-70 showed a narrow distribution pattern with expression initially believed to be restricted to T cells and natural killer cells, although recent reports have found Zap-70 expressed in some populations of normal and malignant B cells [50,51]. It was quickly realized that Zap-70 plays a crucial role in T cell activation and development. Mice deficient in Zap-70 are arrested in T cell development at the double positive stage and demonstrate a complete absence of both $CD4^+$ and $CD8^+$ mature T cells in peripheral lymphoid tissues [52]. Furthermore, several mutations in Zap-70 in humans have been shown to cause severe combined immunodeficiency (SCID) [53–55]; these patients display an absence of $CD8^+$ T cells but an elevated number of $CD4^+$ T cells that fail to signal normally through the T cell receptor. Most mutations in Zap-70 linked to SCID map to the kinase domain [56]. The central role of Zap-70 in TCR signaling together with its narrow expression pattern has focused attention on Zap-70 as an attractive target for treatment of T cell mediated disorders [57].

### 2.3. Tec family kinases

Activation of Tec family tyrosine kinases occurs downstream of both Src and Syk family kinases in signaling pathways (Fig. 1). Upon signal initiation, most Tec family kinases translocate to the plasma membrane through binding of their pleckstrin homology (PH) domain to phosphatidylinositol (3,4,5)-trisphosphate ($PIP_3$) created by the activation of PI 3′-kinase [58,59]. At the membrane Tec family kinases become phosphorylated within their activation loop (Tyr 551 in Btk) via either Src family

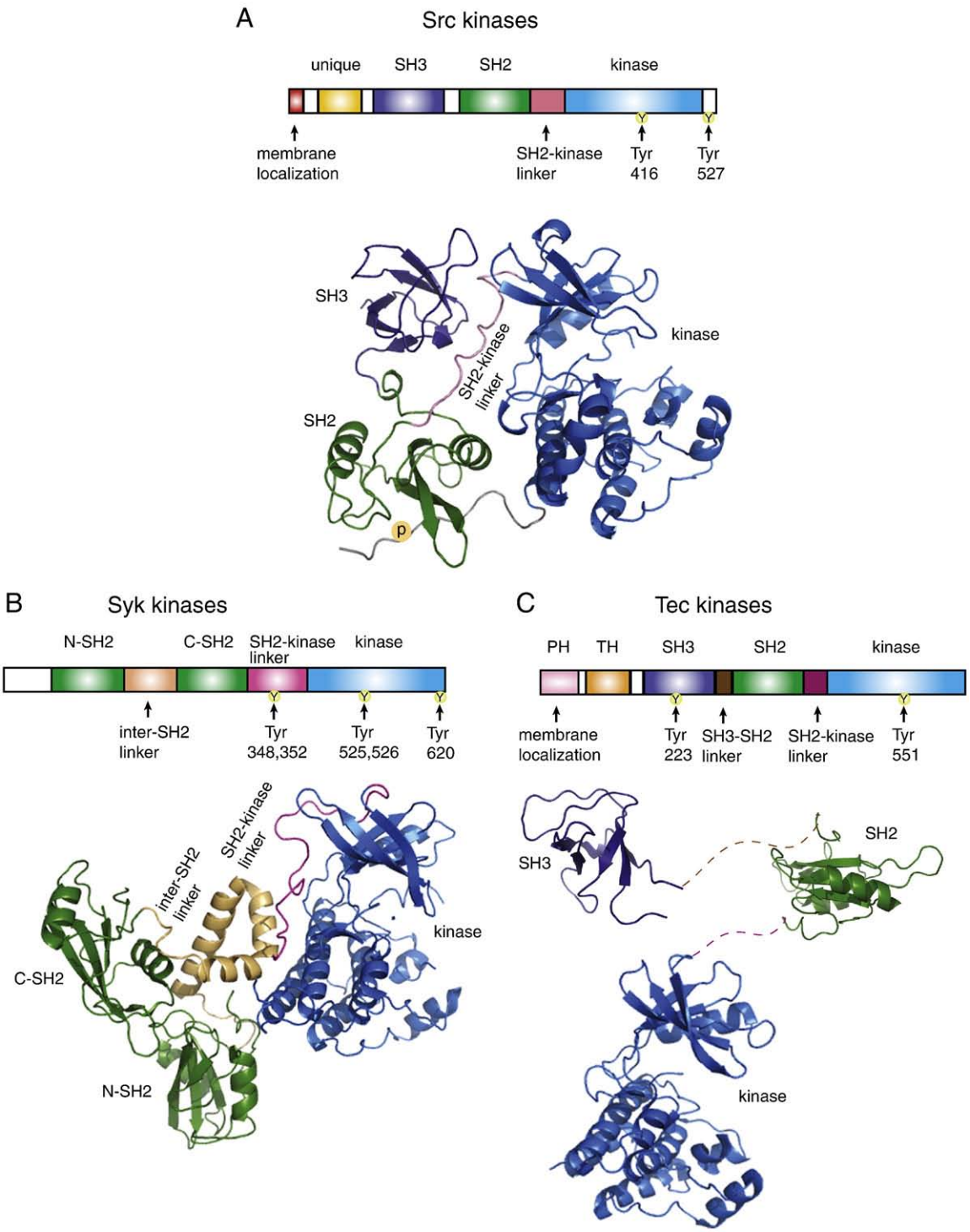


**Fig. 2.** Domain organization and three-dimensional structure of Src, Syk and Tec tyrosine kinases. A) Structure of Src kinases. Important regulatory sites of tyrosine phosphorylation include tyrosines within both the activation loop (Tyr 416 in Src) and C-terminal tail (Tyr 527 in Src). The x-ray structure of Src in an inactive conformation indicates that phosphotyrosine 527 in the C-terminal tail binds the SH2 domain and that the SH2-kinase linker binds the SH3 domain at the back surface of the kinase domain. B) Structure of Syk kinases. Important regulatory sites of tyrosine phosphorylation include residues within the SH2-kinase linker (Tyr 348 and Tyr 352 in Syk), activation loop (Tyr 525 and Tyr 526 in Syk), and C-terminus (Tyr 620 unique to Syk). The x-ray structure of Zap-70 in an inactive conformation indicates that segments of the SH2-kinase linker and inter-SH2 domain linker pack together against the kinase domain and down regulate activity. C) Structure of Tec kinases. Important regulatory sites of tyrosine phosphorylation include residues within the SH3 domain (Tyr 223 in Btk) and activation loop (Tyr 551 in Btk). Structures of individual domains of the Tec kinase Itk have been determined, but as of yet no full length structure of a Tec family kinase has yet been determined. Figures constructed using Pymol (Src pdb: 1fmk; Zap-70 pdb: 2ozo; Itk kinase pdb: 1snu; Itk SH2 pdb: 2etz; Itk SH3 pdb: 2rna).

kinases or autophosphorylation. Another site that becomes phosphorylated is within the SH3 domain of the kinase (Tyr 223 in Btk). The SH2 domain of Tec family kinases has also been reported to bind to a phosphorylated site within adapter molecules such as BLNK in B cells or SLP-76 in T cells [60,61], which may modulate activity and/or properly localize Tec kinases in proximity to their substrates.

The Tec family of kinases consists of 5 different kinases: Bmx, Btk, Itk, Tec, Txk/Rlk [62]. Of these kinases, Btk, Itk, and Tec each share a

conserved domain structure consisting of an N-terminal PH domain, a Tec homology (TH) domain unique to Tec kinases that contains both a "Btk motif" and a proline rich stretch, an SH3 domain, an SH2 domain, and a C-terminal kinase domain (Fig. 2). Bmx is different in structure from other Tec kinases since it demonstrates low homology in its SH3 and TH domain regions [63]. Txk/Rlk has a markedly different domain structure at its N-terminus than the other Tec kinases in that it lacks both a TH and a PH domain but instead contains a region of palmitoylated cysteines that facilitate membrane localization [63].

#### 2.3.1. Btk

Btk (Bruton's tyrosine kinase) was first identified by screening a B cell cDNA library for novel tyrosine kinases [64]. The identified gene was found to be expressed primarily in a variety of hematopoetic cells including B cells, mast cell, and macrophages but not T cells. The human *btk* gene was found to map to the same chromosome region as the gene responsible for a human immunodeficiency disorder known as X-linked agammaglobinemia (XLA) [64,65]; this disorder is characterized by a marked reduction in mature B cells, functionally linking Btk to the B cell maturation process. Further work confirmed that mutations in Btk cause XLA and now more than 600 different mutations in Btk have been observed to result in XLA [66]. A point mutation in Btk was also found to be responsible for murine xid [67], a condition in mice characterized by abnormal B cell function. Btk knock-out mice have a similar phenotype as xid mice [68,69]. In addition to functioning downstream of the B cell receptor, recent studies have also indicated a role for Btk in signaling in B cells through Toll-like receptors [59,70]. Hence, Btk appears to be a generally attractive target for treatment of disorders associated with aberrant B cell signaling such as B cell lymphoma and certain inflammatory disorders [71,72]. It should be noted that while Btk has been most characterized in B cells, it is also important for signaling in mast cells, macrophages, platelets, and osteoclasts.

#### 2.3.2. Itk

Itk (IL-2 inducible T cell kinase) was first cloned by screening T cell lines for previously uncharacterized tyrosine kinases [73]. The identified *itk* gene was found to be expressed predominantly in T cells and to demonstrate an increase in RNA level in response to the cytokine IL-2 [73]. Itk knock-out mice confirmed a key role in T cell maturation [74,75]. A key advance regarding the function of Itk came with the discovery that Itk-deficient $CD4^+$ T cells fail to effectively differentiate into $T_h2$ effector cells [76]. For instance, a key defect of $Itk^{-/-}$ mice is their lack of ability to generate a $T_h2$-mediated defense against invading extracellular parasites and also some viral infections [77]. Given its central role in $T_h2$ cell development, Itk has been under investigation as a target for disorders associated with inappropriate $T_h2$ cell activation such as atopic dermatitis and asthma. Of note, $Itk^{-/-}$ mice exhibit decreased lung infiltration of T cells and reduced lung inflammation in a mouse model of asthma [78]. However, a recent report has noted that $Itk^{-/-}$ mice also show enhanced signaling through γδ T cells [79], which suggests that pharmacological inhibition of Itk may also have undesirable physiological effects such as an enhancement of IgE production.

## 3. Activation of Syk family kinases

In this section, studies investigating the mechanisms regulating Syk family kinase activity are described. Attention is focused on 1) kinase structure, 2) regulation by phosphorylation, 3) regulation by domains outside the kinase domains as well as allosteric ligands, and 4) substrate recognition.

### 3.1. Structure

Structural studies of the isolated kinase domains, the isolated tandem SH2 domains, and the full length proteins have all helped reveal the mechanism of Syk family kinase activation. The structures of the kinase domains of both Syk and Zap-70 in complex with small molecule inhibitors first revealed the architecture of the kinase domains [80–82]. A key feature of these structures was that the kinases were not phosphorylated within the activation loop but nonetheless displayed conformations associated with the activated state of kinases. This suggests that activation loop phosphorylation may not be required to increase the activity of these constructs.

The tandem SH2 domains of Zap-70 have been solved in an unliganded form [83], while the SH2 domains from both Syk and Zap-70 have been determined in complex with dually phosphorylated ITAM peptides [84,85]. These structures, together with several other lines of evidence [86–88], indicate that the tandem SH2 domains have significant regions of disorder in the unliganded state (particularly in inter-SH2 domain region) that become ordered upon binding of ITAM peptides. These findings suggested that ITAM binding can couple to long distance conformational changes within the kinase structure. Interestingly, the tandem SH2 domains of Syk demonstrate conformational flexibility even after binding to ITAM peptides [84], which likely reflects the capability of the Syk tandem SH2 domains, but not the Zap-70 SH2 domains, to bind a wide variety of differently spaced ITAM sequences.

A breakthrough in understanding the activation mechanism of Syk family kinases was the determination of the structure of a full length version of Zap-70 in its inactive conformation [89]. The structure revealed molecular interactions unique to Zap-70 (and possibly Syk) between the inter-SH2 domain, the SH2-kinase linker, and the back of the kinase domain that distort the Zap-70 kinase domain into an inactive conformation. Based on this structure, either binding of phosphorylated ITAM sequences to the kinase or phosphorylation within the SH2 domain-kinase linker should disrupt autoinhibitory interactions within the linker-kinase sandwich and result in kinase activation. This hypothesis has been supported by biochemical and mutagenesis studies [90,91].

### 3.2. Regulation by phosphorylation

A major mechanism for regulating Syk family kinases is via changes in phosphorylation. This is apparent from multiple lines of evidence and has recently been illustrated through enzymatic studies [91,92]. In these experiments, both Syk and Zap-70 that are pre-phosphorylated display an initial linear time course of product formation, consistent with an active kinase conformation. In contrast, either Syk or Zap-70 that is dephosphorylated prior to analysis show a profound lag phase in product formation. This indicates that the kinase begins the experiment in a low activity state and subsequently activates via autophosphorylation. The difference in catalytic rate between high and low activity states is ~100-fold. Interestingly, Syk and Zap-70 display a different ability to self-activate, with Syk demonstrating a much shorter lag phase than Zap-70 [91]. Hence, Syk may either be a better substrate for itself than Zap-70 or else may be less stabilized in its inactive conformation. Incubation of either Syk or Zap-70 with its upstream Src family kinase (Lyn or Lck, respectively) results in rapid conversion to a fully active conformation, confirming the ability of Src kinases to activate Syk family kinases.

While the enzymatic studies described above demonstrate the significance of phosphorylation for Syk family kinase activity, they do not distinguish the sites that are important. Several phosphorylation sites in Syk kinases have been identified [93]. These include 2 Tyr residues within the activation loop (Y525, Y526 for Syk; Y519, Y520 for Zap-70), 2 Tyr residues within the SH2-kinase linker (Y348, Y352 for Syk; Y315, Y319 for Zap-70), and Tyr residues near the C-terminus of Syk (including Y630; this Tyr is not present in Zap-70). Mutagenesis studies have helped define the role of these residues in Syk family kinase activation. For instance, mutation of the activation loop tyrosines for both the Syk and Zap-70 isolated kinase domain does

not result in any reduction in activity [80,94]; this is consistent with the structures that showed an active conformation despite the absence of phosphorylation [80,81]. In contrast, deletion mutagenesis within the SH2 domain-kinase linker highlighted the segment containing Tyr 348 and Tyr 352 as important [91]. Furthermore, point mutations in Tyr 315 and Tyr 319 of Zap-70 [89,95], as well as Trp 131 in the inter-SH2 domain linker [90], were shown to profoundly modulate Zap-70 activation. For Syk, phosphorylation of Tyr 630 within the tail of the protein also seems capable of disrupting key autoinhibitory interactions [96]. These mutational studies all point to disruption of the linker-kinase sandwich as important for Syk family kinase activation.

### 3.3. Regulation by protein modules and allosteric ligands

Studies comparing the activity of different length constructs of Syk kinases have corroborated the model that disrupting the kinase-linker sandwich is important to activation. For instance, the isolated kinase domain of Syk demonstrates 10-fold greater maximal activity than activated full length enzyme [94], indicating domains outside the kinase domain repress activity. Furthermore, like phosphorylation of the SH2-kinase linker, binding of ITAM peptides to both Syk and Zap-70 causes full activation of the enzyme [91]. Based on this finding, Syk was described as an "OR-gate switch" since either phosphorylation OR ITAM binding is sufficient to cause complete enzymatic activation [91] (see Section 5).

The finding from enzymatic studies that ITAM binding fully activates Syk kinases corroborated structural studies indicating ITAM binding causes a large conformational change and motivated the use of binding methodologies to further study the tandem SH2 domain-ITAM peptide interaction. Both surface plasmon resonance and enzymatic assays indicate that dually phosphorylated ITAM peptides bind full length Syk with an affinity in the tens of nanomolar range [91]. This level of potency was corroborated by studies utilizing fluorescence quenching that were performed with the isolated tandem SH2 domains of Syk [97–99]. Thermodynamic studies also indicated a significant ordering of the tandem SH2 domain structure accompanied ITAM binding [97–99], supporting data from structural studies.

### 3.4. Substrate recognition

While a mechanistic understanding of how phosphorylation and ITAM binding regulate Syk kinase activity has begun to emerge, a less clear picture of how Syk kinases recognize protein substrates is currently available. Nonetheless, some aspects of substrate recognition have been characterized. For instance, mapping the substrate selectivity of Syk revealed a preference for negatively charged amino acids flanking the catalytic tyrosine [100]; peptides containing this motif have been useful substrates for measuring Syk kinase activity [101,102]. Recently, substrate peptides that also have excellent selectivity for Syk have been identified. Upon incubation of these peptides with B cell lysates containing numerous different kinases, Syk was the only kinase that demonstrated phosphorylation of the peptides [91,103]. Does binding of peptide substrates to Syk kinases affect the affinity of ATP? The answer appears to be no because the steady state kinetic mechanism of Syk revealed no cooperative interactions between substrate and ATP binding sites [94]. Steady state kinetic studies also revealed a surprising lack of inhibitory potency for substrate-analog inhibitors that contain Phe substituted for Tyr [94]. This finding suggests that Syk employs a "substrate clamping" kinetic mechanism where the potency of the substrate is significantly less than the value of the peptide Km [104]. Finally, while substrate docking sites distant from the kinase active site have recently been identified for several tyrosine kinase families including Csk and Tec kinases, no substrate docking site has yet been identified for the Syk family of kinases.

## 4. Activation of Tec family kinases

The Tec family of kinases shows similarities and differences with regard to activation in comparison to the Syk family kinases described above. This section discusses the activation of Tec kinases focusing on 1) protein structure, 2) phosphorylation sites, 3) interactions between the protein domains of the kinase, and 4) recognition of substrates.

### 4.1. Structure

Multiple structures of the individual domains of Tec family kinases (i.e., PH, SH3, SH2, and kinase) have been determined [105–116]. These structures have provided insight into several of the factors that modulate Tec kinase activation including membrane localization of the kinase and regulatory domain interactions. For instance, the structure of the Btk PH domain revealed a positively charged surface that binds inositol phospholipids with high affinity, providing a molecular understanding for kinase activation via plasma membrane translocation [105–107]. Structures of the isolated kinase domains of both Btk and Itk have also been published; the Btk structure demonstrated a catalytically incompetent conformation even though the activation loop did not occlude the active site [115,116].

While the structures of the PH domain and kinase domain described above provide some insight into Tec kinase activation, they did not shed light on how domains within Tec family kinases interact to impact activity. In contrast, several NMR structures of the Itk SH2 and SH3 domain have given information on how these domains interact with other Itk domains and each other to regulate activation. For instance, the Itk SH3 domain structure was solved together with a polyproline sequence from the TH domain [108], showing how this inter-domain interaction may regulate self-association of Itk. A separate structure of the Itk SH3 domain bound to the Itk SH2 domain demonstrated that the SH3 domain ligand binding site can be occluded by the SH2 domain, which could block Itk activation through preventing ligand binding to the SH3 domain [117]. Interestingly, interaction between the SH3 and SH2 domain is favored when a proline residue in the SH2 domain (Pro 287) undergoes isomerization into its *cis* conformation. This isomerization of Pro 287 was shown to occur in a previous structure of the Itk SH2 domain and also was shown to be functionally important for regulating Itk activity [118].

Unlike Syk family kinases, no full length structure for a Tec family kinase is yet available to provide a more solid understanding into how interdomain interactions regulate Tec family activation. Tec kinases do share a common sequential arrangement of the SH3 domain, SH2 domain, and kinase domain with both Src kinases and Csk, suggesting the possibility of structural similarity with one of these kinase families. Full length structures of both Src and Csk have been determined revealing that these 2 kinases have a much different orientation of the SH3 and SH2 domains from one another [26,27,119]. Biochemical data suggest that the Tec family kinases may share greater similarity with Csk, but convincing insight into Tec structure will be obtained only following structure determination of a Tec kinase itself.

### 4.2. Regulation by phosphorylation

Two key sites of regulatory phosphorylation for Tec kinases have been identified in cells: a site within the activation loop (Btk Tyr 551, Itk Tyr 511) and a site within the SH3 domain (Btk Tyr 223, Itk Tyr 180) [120–123]. Does phosphorylation of either of these sites (or both) directly alter the enzymatic activity of Tec kinases, or is regulation via another mechanism? Studies monitoring the enzymatic activity of Tec kinases *in vitro* have recently addressed this question quantitatively.

With regard to the activation loop tyrosine, convincing evidence indicates that phosphorylation directly increases kinase activity [124,125]. For instance in enzymatic studies with Btk, activation loop phosphorylation increased activity 10-fold, and a Y551F mutant

demonstrated 10-fold lower maximal activity than wild type Btk [125]. Within cells this activation loop site is widely believed to be phosphorylated by Src family kinases. However enzymatic studies with Btk indicate that Btk can autophosphorylate at Tyr 551. In these studies, the time course of product formation by initially dephosphorylated Btk demonstrates a distinct lag phase, consistent with an autophosphorylation-dependent increase in activity [125]. In cells, at least one report has attributed Btk phosphorylation at Tyr 551 to both Src kinases and autophosphorylation [126], indicating a cellular significance to the Btk autophosphorylation at Tyr 551 observed *in vitro*.

While activation loop phosphorylation is widely believed to increase Tec family kinase activity, the impact of phosphorylation within the SH3 domain on kinase activity is modest. For instance, mutation of Itk Tyr 180 to Phe affected the kinetic parameters of Itk by 2-fold or less [127]. Mutation of Itk Tyr 180 or Btk Tyr 223 has also been observed to have little effect on maximal kinase activity in cells [120,122]. The functional significance of SH3 domain phosphorylation in cells is more likely to result from modulation of protein–protein interactions than effects on kinase activity [128]. Specifically, Tyr 180 in Itk is located at the edge of the SH3-polyproline binding pocket and autophosphorylation at this site has been shown to diminish affinity of the SH3 domain for polyproline containing ligands [127,129]. Examination of the mechanism of Itk Tyr 180 autophosphorylation has indicated that it occurs in an intramolecular fashion [127].

### 4.3. Regulation by protein modules and allosteric ligands

The isolated kinase domains of Tec family kinases demonstrate low intrinsic activity, suggesting that domains outside of the kinase domain may be required together with phosphorylation for maximal activity. Truncation mutagenesis studies with Itk have addressed this issue and clearly shown that the SH2-kinase linker is essential for high Itk activity [130]. In this study, it was determined that a conserved Trp residue (Trp 355 in Itk, Trp 395 in Btk) within the linker docks against the kinase domain and helps properly position residues of the kinase domain for catalysis. However, if residues of the SH2-kinase linker (including the conserved Trp) are present, will a truncated Tec kinase demonstrate as high of activity as the full length protein? Enzymatic studies with Btk indicated that a construct containing just the kinase domain and a portion of the linker (including Trp 395) showed low intrinsic activity in the dephosphorylated state but following activation loop phosphorylation by Lyn became nearly as active as full length Btk [124]. Together, these findings are consistent with a dual requirement for Tec family activation: activation loop phosphorylation and proper interactions between the SH2-kinase linker and the kinase domain. While it is clear that phosphorylation of the activation loop is actively regulated, it has not yet been established whether docking of the SH2-kinase linker against the kinase domain is also dynamically regulated or else constitutively positioned to facilitate activation. It is possible that Tec family SH2 domains also participate in properly positioning the SH2-kinase linker, which would be analogous to the recently shown role of the SH2 domains in facilitating the activity of the Fes and Abl kinases [131].

Does binding of the protein modules of Tec kinases (the SH2, SH3, and PH domains) to their cognate ligands (pTyr residues, polyproline sequences, and inositol phospholipids) provide an additional way to increase kinase activity? It does not appear to be the case. It has not yet been reported that binding of allosteric ligands to Tec kinases increases kinase activity *in vitro*. Furthermore, incubation of Btk with ligands for its PH, SH2, and SH3 domains failed to significantly modulate its enzymatic activity. Hence, Tec family kinases may be distinct from both Syk and Src family kinases in which binding of ligands to the SH2 and/or SH3 domains of these kinases can increase kinase activity.

### 4.4. Substrate recognition

Tec family kinases have been shown to utilize a unique substrate recognition mechanism in order to achieve full activation *in vivo*. Specifically, Itk employs a substrate docking site to facilitate autophosphorylation within its SH3 domain as well as phosphorylation of downstream substrates such as phospholipase Cγ1 [132]. The substrate docking site involves interactions between regions in the kinase domain outside the kinase active site with an SH2 domain in the substrate that is located proximal to the catalytic tyrosine residue. The residues in the Itk SH2 domain that interact with the kinase to facilitate docking and autophosphorylation have been mapped to 3 specific loops in the structure (AB, EF, and BG) together with part of a β-strand (βD) [133]; a distinct set of basic residues facilitates binding of the PLCγ1 SH2 domain to Itk [134]. Tec family kinases are only the second example of tyrosine kinases utilizing docking sites to facilitate target phosphorylation: a docking mechanism has also been characterized for Csk [135–137]. However, substrate docking sites are more well-established in serine/threonine kinases, and further investigation of substrate recognition by different families of tyrosine kinases may yet reveal new docking sites that facilitate kinase activation.

## 5. Src, Syk, and Tec kinases as distinct types of molecular switches

As mentioned in the Introduction, kinases often function at nodes in signaling pathways [3–6]. The signaling output downstream from a kinase node is determined by the nature of the kinase switch at that node, and there are known to be several varieties of kinase switches at nodes [138]. One type is a "graded" switch. This type of switch shows an incremental increase in kinase activity with an increasing number of activating stimuli (See Fig. 3A). A "graded" kinase switch is different from an "all-or-none" kinase switch that demonstrates only 2 levels of responses: inactivity or full activity [139–141] (See Fig. 3B and C). An "all-or-none" kinase switch can be further subclassified as either an "OR-gate" or an "AND-gate" switch depending on whether a single stimulus (OR-gate) or multiple stimuli (AND-gate) are required for activation [138]. Whether a kinase demonstrates "graded", "OR-gate", or "AND-gate" switching is determined by molecular features of the kinase. The accumulation of data regarding Src, Syk, and Tec family kinases now makes it possible to classify each as a particular type of switch.

The Src family of kinases was the first nonreceptor tyrosine kinase family to be well characterized [23–25]. Src kinases utilize a rich variety of mechanisms to regulate activity [142]. Both phosphorylation of the activation loop (Src Tyr 416) and dephosphorylation of the C-terminal tail (Src Tyr 527) enhance activity [25,28]. Binding of exogenous ligands to both the SH2 domain and SH3 domain also disrupt autoinhibitory interactions and result in activation [30,31,143]. How are these processes integrated to regulate Src activity overall? By isolating each activation step and studying it individually, activation of Src kinases was found to be a sequential process [30]; full activity is only achieved if all activating stimuli are present. Based on these findings, Src kinase activation appears to be best classified as a "graded" type of switch.

For Syk kinases, the major factor regulating activity is intermolecular interactions forming the linker-kinase sandwich [89–91]. Disruption of these interactions via phosphorylation of the SH2-kinase linker results in activation. ITAM binding to the tandem SH2 domains also disrupts these interactions, allowing kinase activation. Phosphorylation of other residues in the vicinity of the linker-kinase sandwich (such at Tyr 630 in Syk) also perturbs autoinhibitory interactions [96]. In contrast, phosphorylation within the activation loop appears to play a lesser role in enhancing activity than in other tyrosine kinase families [80,94]. Comparing the level of activation that occurred via autophosphorylation and ITAM binding indicated that each process by itself was sufficient to fully activate the enzyme [91]. Based on this finding, activation of Syk family kinases is an "OR-gate" switch.

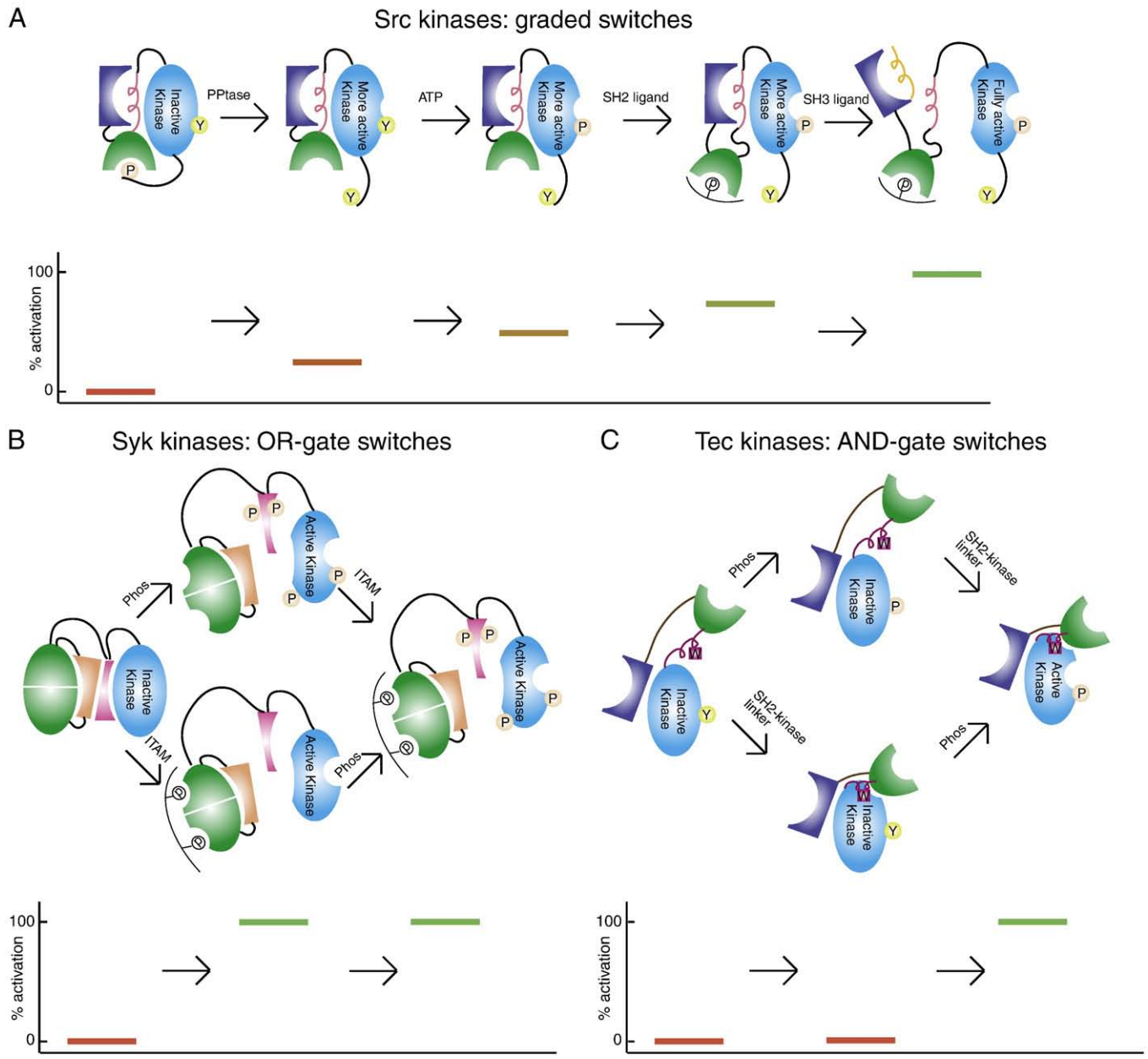


**Fig. 3.** Src, Syk, and Tec kinases as molecular switches. A) Model of Src kinase activation as a graded switch. It has been shown that Src is incrementally activated by several events including (from left to right) dephosphorylation of the C-terminal tail, phosphorylation of the activation loop, binding of a ligand to the SH2 domain, and binding of ligands to the SH3 domain. Each additional event has been reported to result in a higher level of Src kinase activity. B) Model of Syk kinase activation as an "OR-gate" switch. It has been reported that Syk is activated in an all-or-none fashion after either phosphorylation within the SH2-kinase linker or binding of phosphorylated ITAM peptides. The presence of both stimuli does not further enhance Syk kinase activity. C) Model of Tec kinase activation as an "AND-gate" switch. The available data for Tec kinases suggest that both activation loop phosphorylation and proper docking of the SH2-kinase linker against the kinase are required for high activity.

For Tec family kinases, disruption of autoinhibitory interactions plays a lesser role in activation than for Src and Syk kinases. Instead, phosphorylation of the activation loop tyrosine is critical for activation [124,125]. Furthermore, positioning of the conserved Trp (Trp 355 in Itk) within the SH2-linker region into its pocket in the kinase domain is central to achieving high activity [130]. Interestingly, neither activation loop phosphorylation nor positioning of the SH2 kinase linker is sufficient by itself to strongly active Tec kinases. Hence, Tec kinases appear to be best classified as "AND-gate" switches since multiple events (activation loop phosphorylation and positioning of the SH2-kinase linker) are necessary for full activation.

The biological role of Src, Syk, and Tec family kinases in signaling cascades appears to be largely determined by the type of switching (graded, OR-gate, or AND-gate) demonstrated by each kinase family. For instance, the graded response demonstrated by the Src kinases likely permits its dual role of activating molecules that have both a positive (at low stimulus levels) and negative (at high stimulus levels) role in signaling [15,19]. The OR-gate switch demonstrated by Syk kinases provides redundancy in activation and explains how Syk activity is maintained over time after only transient activation of cell surface receptors [144]. Finally, the Tec kinases appear to display tighter regulation than the Src or Syk kinases due to their AND-gate activation mechanism.

Overall, a picture emerging from recent studies is that, despite similarities in domain organization, the Src, Syk and Tec kinases have evolved unique strategies to regulate their enzymatic activities. An

understanding of the molecular mechanisms unique to each kinase family began to emerge only after detailed mechanistic, structural, and biochemical studies.

## 6. Conclusion

This review described the molecular mechanisms regulating enzymatic activity for the Src, Syk, and Tec family of kinases. These kinase families are essential for signaling immediately downstream of antigen and Fc receptors in haematopoietic cells. New structural and biochemical studies have now provided a greater understanding of the factors involved in regulating the activity of these kinases. The current data are consistent with a model of Src kinase activation as a graded switch, a model of Syk kinase activation as an OR-gate switch, and a model of Tec kinase activation as an AND-gate switch (Fig. 3). These distinct activation mechanisms are essential to the biological role of these kinases in cell signaling processes. For additional information on the kinases described here, the reader is referred to other excellent, recent reviews [57,59,93,128].

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