

# Phylogeny of Tec Family Kinases: Identification of a Premetazoan Origin of Btk, Bmx, Itk, Tec, Txk, and the Btk Regulator SH3BP5

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## ABSTRACT

It is generally considered mammals and birds have five Tec family kinases (TFKs): Btk, Bmx (also known as Etk), Itk, Tec, and Txk (also known as Rlk). Here, we discuss the domains and their functions and regulation in TFKs. Over the last few years, a large number of genomes from various phyla have been sequenced making it possible to study evolutionary relationships at the molecular and sequence level. Using bioinformatics tools, we for the first time demonstrate that a TFK ancestor exists in the unicellular choanoflagellate *Monosiga brevicollis*, which is the closest known relative to metazoans with a sequenced genome. The analysis of the genomes for sponges, insects, hagfish, and frogs suggests that these species encode a single TFK. The insect form has a divergent and unique N-terminal region. Duplications generating the five members took place prior to the emergence of vertebrates. Fishes have two or three forms and the platypus, *Ornithorhynchus anatinus*, has four (lacks Txk). Thus, not all mammals have all five TFKs. The single identified TFK in frogs is an ortholog of Tec. Bmx seems to be unique to mammals and birds. SH3BP5 is a negative regulator of Btk. It is conserved in choanoflagellates and interestingly exists also in nematodes, which do not express TFKs, suggesting a broader function in addition to Btk regulation. The related SH3BP5-like protein is not found in Nematodes. © 2008, Elsevier Inc.

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## I. TYROSINE KINASES AND THE TEC FAMILY

### A. Identification and characteristics of TFKs

In mammals, Tec family kinases (TFKs), Btk, Bmx (Etk), Itk, Tec, and Txk (Rlk), form the second largest family of cytoplasmic protein-tyrosine kinases (PTKs), the largest being related to Src, harboring nine Src family kinases (SFKs) (Caenepeel *et al.*, 2004; Quintaje and Orchard, 2008). We have used the official Human Genome Nomenclature Committee (HGNC) (<http://www.genenames.org/>) gene and protein names throughout the text. Human TFKs are in upper case and gene names in italics. The 2008 annotation lists 480 classical and 24 nonclassical protein kinases in man, out of which 90 are PTKs, while mice have

93 tyrosine kinases (Quintaje and Orchard, 2008). The protein kinases constitute one of the largest mammalian gene families comprising about 2% of all genes or about 10% of signaling functions coding genes.

With the exception of Txk, TFKs are characterized by an N-terminal pleckstrin homology (PH) domain, followed by a Tec homology (TH), Src homology (SH) -3, -2, and -1 (catalytic) domains (Smith *et al.*, 1994b; Vihinen *et al.*, 1994a). Txk instead has a cysteine-rich string, which, like PH domains, is required for temporary membrane attachment. PH domains in TFKs are also involved in binding to heterotrimeric G proteins and protein serine/threonine kinases (PSKs).

The TH domain consists of an N-terminal Zn<sup>2+</sup>-binding Btk motif (Hyvönen and Saraste, 1997; Vihinen *et al.*, 1994a, 1997a) and one or two proline-rich motifs (Smith *et al.*, 2001; Vihinen *et al.*, 1994a, 1997a). Bmx lacks the typical proline-rich region (PRR) of the TH domain and also has an altered SH3 domain (Smith *et al.*, 2001; Tamagnone *et al.*, 1994). The PRRs participate in inter- and intramolecular SH3 domain binding. The SH2 and SH3 domains are docking modules, which bind to polyproline helices and phosphotyrosines, respectively. The kinase domain is the only catalytic entity in TFKs.

The mammalian TFKs were cloned during 1990–1995 (Haire *et al.*, 1994; Heyeck and Berg, 1993; Hu *et al.*, 1995; Mano *et al.*, 1990; Robinson *et al.*, 1996; Siliciano *et al.*, 1992; Tamagnone *et al.*, 1994; Tsukada *et al.*, 1993; Vetric *et al.*, 1993; Yamada *et al.*, 1993) and immediately received wide interest, especially owing to the fact that BTK mutations cause an X-linked form of B lymphocyte deficiency (X-linked agammaglobulinemia, XLA) in man (Lindvall *et al.*, 2005; Tsukada *et al.*, 1993; Väliäho *et al.*, 2006; Vetric *et al.*, 1993; Vihinen *et al.*, 1995a) and X-linked immunodeficiency (XID) in mice (Rawlings *et al.*, 1993; Thomas *et al.*, 1993). Btk is expressed in lymphoid cells but absent from T cells and plasma cells (Smith *et al.*, 1994a).

Disease-related mutations affecting the corresponding enzyme in T lymphocytes, ITK, has to date not been reported, but this gene resides on an autosome making any loss-of-function phenotype considerably less common than that observed for BTK. However, Itk plays an essential role in T-lymphocyte development as shown by knocking out the gene in mice (Liao and Littman, 1995). The gene was initially identified using degenerate primers to amplify cDNA from an IL-2-dependent mouse T-cell line (Siliciano *et al.*, 1992) or from neonatal mouse thymus (Heyeck and Berg, 1993).

Tec was cloned from a hepatic carcinoma (Mano *et al.*, 1990), but was later found to be expressed in several tissues, including B lymphocytes (Mano *et al.*, 1993). The phenotype of mice with Btk deficiency is much milder than the one seen in humans. While Tec is also expressed in human B lymphocytes, in mice the generation of double knockouts for Btk and Tec causes an XLA-like

phenotype, whereas Tec single-knockout mice do not have an overt phenotype (Ellmeier *et al.*, 2000). An osteoclast defect has been reported both in isolated Btk deficiency (Lee *et al.*, 2008) and in combined Btk/Tec deficiency (Shinohara *et al.*, 2008). More subtle effects of Tec have been recognized in platelets (Atkinson *et al.*, 2003; Crosby and Poole, 2002; Oda *et al.*, 2000), erythroid (Schmidt *et al.*, 2004a; van Dijk *et al.*, 2000), and phagocytic cells (Jongstra-Bilen *et al.*, 2008; Melcher *et al.*, 2008). In these cells, Tec is important for signaling through various receptors (Atkinson *et al.*, 2003; Crosby and Poole, 2002; Oda *et al.*, 2000; Schmidt *et al.*, 2004b; van Dijk *et al.*, 2000), and lack of Tec resulted in increased levels of caspases (Melcher *et al.*, 2008). Furthermore, Tec showed a unique late effect in the phagocytic process (Jongstra-Bilen *et al.*, 2008).

Bmx was originally identified from a bone marrow-derived cDNA library (Tamagnone *et al.*, 1994) and was later found to be expressed mainly in endothelial cells as well as in prostate tumors (Ekman *et al.*, 1997; Robinson *et al.*, 1996). Loss-of-function mutations in humans have not been detected, which is somewhat unexpected owing to its X-chromosomal location and the viability of the mouse knockout. Such mice are characterized by defects in arteriogenesis and angiogenesis (He *et al.*, 2006; Rajantie *et al.*, 2001).

Txk was first identified from human peripheral blood and murine thymus cDNA libraries, respectively (Haire *et al.*, 1994; Hu *et al.*, 1995), and was later found to be mainly involved in T-lymphocyte development, since *Itk*<sup>-/-</sup> and *Txk*<sup>-/-</sup> mice have a more severe phenotype as compared to an *Itk*<sup>-/-</sup> single defect (Broussard *et al.*, 2006; Gomez-Rodriguez *et al.*, 2007; Schaeffer *et al.*, 1999). Recently a role for Txk has also been described in NKT-cell development (Felices and Berg, 2008).

## B. Biological functions of TFKs

The TFKs have been implicated as pivotal components of signaling pathways downstream of extracellular receptor stimuli, such as lymphocyte antigen receptors (Felices *et al.*, 2007; Lindvall *et al.*, 2005; Schmidt *et al.*, 2004a,b). A functional defect of *Itk* and *Btk* kinases affect both innate and adaptive immunity in T cells (Berg *et al.*, 2005) and B cells (Brunner *et al.*, 2005; Hasan *et al.*, 2008; Mansell *et al.*, 2006), respectively. Activation of TFKs is a two-step event, which requires phosphorylation by a SFK member, and translocation to the plasma membrane, mediated by the PH domain (Lewis *et al.*, 2001; Nore *et al.*, 2000; Varnai *et al.*, 2005). It is not known which of these comes first, but presumably membrane translocation is the initial event, since *Btk* mutants

lacking membrane-binding activity are not phosphorylated. Moreover, SFKs are known to mainly reside in the membrane (Ingley, 2008). In the case of Txk, which lacks PH domain, N-terminal palmitoylated cysteine-string motif is responsible for membrane colocalization.

Btk seems to have a dual role in apoptosis, under certain conditions being protective, while in other cellular contexts it instead induces apoptosis (Islam and Smith, 2000; Uckun, 1998). Bmx was reported to protect cells from apoptosis (Xue *et al.*, 1999). The antiapoptotic role of TFKs may involve AP-1 signaling (Altman *et al.*, 2004). Another potentially antiapoptotic pathway is through NF- $\kappa$ B, which is downstream of Btk (Bajpai *et al.*, 2000; Petro *et al.*, 2000). Recently was shown that NF- $\kappa$ B acts on the Btk promoter region by feedback activation (Yu *et al.*, 2008).

One common phenomenon in TFK-mediated activation is that these kinases modulate actin polymerization and dynamics, which play a central role in cytoskeleton processes including cell division, motility, cell shape, and chemotaxis (Finkelstein and Schwartzberg, 2004; Gomez-Rodriguez *et al.*, 2007; Nore *et al.*, 2000). Btk29A in *Drosophila* is essential for head involution during embryogenesis and also for ring canal growth (Guarnieri *et al.*, 1998; Roulier *et al.*, 1998). Recent studies show that Btk29A controls actin remodeling (Chandrasekaran and Beckendorf, 2005) and microfilament contraction during embryonic cellularization (Thomas and Wieschaus, 2004). It is also of interest to note that the phenotype of flies lacking only the full-length isoform of Btk29A is not lethal (Baba *et al.*, 1999). Instead, the development of male genitals and longevity are affected. The phenotype of TFK-deficient, more primitive species, Porifera (sponge) and choanoflagellate, is not known.

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## II. AIM OF THIS REVIEW

A few reports have discussed the evolution of TFKs (Baba *et al.*, 1999; Cetkovic *et al.*, 2004; Guarneri *et al.*, 1998; Haire *et al.*, 1997, 1998; Nars and Vihinen, 2001; Roulier *et al.*, 1998; Smith *et al.*, 2001), but there are no recent publications related to this topic. Owing to the many genomes characterized over the last years, it seemed timely to compile and analyze the existing data.

SH3BP5 (also called Sab and in flies Parcás) has been identified as a negative regulator of at least Btk in both mammals and *Drosophila melanogaster* (Hamada *et al.*, 2005; Matsushita *et al.*, 1998; Sinka *et al.*, 2002). For this reason the phylogeny of this regulator was also investigated. The emergence and functions of TFKs as well as SH3BP5 are discussed from an evolutionary point of view.

### III. THE FUNCTION OF INDIVIDUAL DOMAINS IN TFKs

#### A. Function of the SH3–SH2–kinase domain complex

Apart from TFKs, the SH3–SH2–kinase domain organization is common to most cytoplasmic tyrosine kinases, namely the Src, Brk/Srm/Frk, Csk, and the Abl families (Mattsson *et al.*, 1996; Serfas and Tyner, 2003), while the Syk/Zap family lacks an SH3 domain and has two SH2 domains and the Fes/Fer family has a single SH2 module located N-terminally to the kinase domain. Jak, Fak, and Ack cytoplasmic tyrosine kinases lack SH3 and SH2 domains. New kinases have emerged by simultaneous duplication of all the three domains together (Nars and Vihinen, 2001).

Owing to that the function of SH3, SH2, and kinase (SH1) domains has been widely investigated and reviewed (Lappalainen *et al.*, 2008; Pawson and Scott, 2005; Pawson *et al.*, 2001; Seet *et al.*, 2006; Williams and Zvelebil, 2004) and since these entities are not unique for TFKs, we will not discuss them in detail, but simply make a few remarks regarding TFK-related properties.

To date there are structures for the SH3 domain of Btk (Hansson *et al.*, 1998), Itk (Andreotti *et al.*, 1997; Laederach *et al.*, 2002, 2003; Severin *et al.*, 2008), and Tec (Pursglove *et al.*, 2002). The thermal unfolding pattern of different TFK SH3 domains has also been analyzed (Knapp *et al.*, 1998). In contrast to most other SH3 domains, the TFK family seems to be regulated by tyrosine phosphorylation as demonstrated for Btk (Park *et al.*, 1996) and Itk (Hao and August, 2002; Wilcox and Berg, 2003) as well as Tec and Bmx (Nore *et al.*, 2003). In Btk, Y223 is in addition to autophosphorylation (Park *et al.*, 1996) target also for Abl (Bäckesjö *et al.*, 2002).

Since SH3 domains bind proline-rich stretches, it is interesting to note that Btk, Itk, and Tec have conserved PRRs in the adjacent TH domain that could bind to the SH3 domain. The Btk motif structure has been solved for BTK together with PH domain (Hyvönen and Saraste, 1997), and the entire TH domain for BMX and ITK [Protein Data Bank (PDB) codes 2ys2 and 2e61, respectively]. Bmx lacks both a typical SH3 domain and the PRR. The fact that both stretches are altered in Bmx is compatible with a functional relationship between a proline-rich, or a polyproline-like region, and the SH3 domain, as manifested in Btk (Hansson *et al.*, 2001a,b; Laederach *et al.*, 2002), Itk (Andreotti *et al.*, 1997; Brazin *et al.*, 2000; Hao and August, 2002; Márquez *et al.*, 2003) as well as in SFKs (Moarefi *et al.*, 1997; Sicheri *et al.*, 1997; Wang *et al.*, 2007; Williams *et al.*, 1997; Xu *et al.*, 1997). The PH and TH domains are missing from Txk.

In the case of Btk there are two PRRs in the TH domain (Smith *et al.*, 2001; Vihinen *et al.*, 1994a) making it possible to form multiple interactions, both intra- and intermolecular in origin (Hansson *et al.*, 2001a,b; Laederach

*et al.*, 2002; Okoh and Vihinen, 2002). This is not the case for Itk, which has a single proline-rich stretch, while for Txk the interaction is mainly intermolecular due to the short connecting linker (Laederach *et al.*, 2002, 2003). However, most of these studies were performed using constructs expressing only the PRR in combination with the SH3 domain. In the single study where a full-length TFK was studied, there was no indication of TH–SH3 interactions (Márquez *et al.*, 2003). As many multidomain proteins, including TFKs, have several overall protein folds depending, for example, on posttranslational modifications such as phosphorylation, or ligand binding, the intramolecular interactions vary. Kinases are known to have several such conformational changes due to active site loop phosphorylation, upper lobe twist because of ATP binding, and C-terminal phosphorylation in regulation of SFKs etc. Recently, Joseph *et al.* (2007a) presented evidence for an intramolecular *cis* mechanism for the phosphorylation of tyrosine 180 in the Itk SH3 domain.

NMR structural studies combined with mutational analysis demonstrated a proline-dependent conformational switch within the Itk SH2 domain. This switch regulates substrate recognition (Brazin *et al.*, 2000). *Cis*–*trans* isomerization of a single prolyl-imide bond (D286–P287) within the SH2 domain influenced substrate recognition (Breheny *et al.*, 2003; Mallis *et al.*, 2002). In Btk the corresponding protein has only *trans* conformation (Huang *et al.*, 2006). Originally, the BTK SH2 domain was modeled (Vihinen *et al.*, 1994b) and used to analyze structure–function relationships along with biophysical methods for selected mutants (Mattsson *et al.*, 2000). The structural consequences of all reported SH2 domain mutations in altogether 10 diseases have been investigated with bioinformatics methods (Lappalainen *et al.*, 2008). *Cis*–*trans* isomerization instead seems to take place within the Btk PH domain (Yu *et al.*, 2006) as described below.

The linker between the SH2 and kinase domain in Itk was recently shown to positively regulate catalysis (Joseph *et al.*, 2007b) and the SH2 domain seems to be involved in substrate binding, not only in Itk, but also in Btk and Tec (Joseph *et al.*, 2007c).

The kinase domain structure has been solved for BTK (Mao *et al.*, 2001) and ITK (Brown *et al.*, 2004). These domains are highly conserved in all TFK sequences.

## **B. Regulation of PH domain binding by phosphoinositide 3-kinase (PI3K)**

The TFKs are the only tyrosine kinases having a PH domain. Likewise, the TH domain is unique to this family. While functional analyses of individual TFKs have demonstrated unique features, they also have many common characteristics. Activation of PI3K generates phosphatidylinositol-3,4,5-trisphosphate

(PIP<sub>3</sub>) serving as a membrane docking site for the PH domain of TFKs. PIP<sub>3</sub> is the most negatively charged plasma membrane lipid, concentration of which can increase 40-fold within seconds after PI3K activation (Stephens *et al.*, 1993). PIP<sub>3</sub> binding characterizes the PH domain of Btk (Manna *et al.*, 2007; Nore *et al.*, 2000; Rameh *et al.*, 1997; Salim *et al.*, 1996; Watanabe *et al.*, 2003), Bmx (Ekman *et al.*, 2000; Jiang *et al.*, 2007; Qiu *et al.*, 1998), Itk (August *et al.*, 1997; Huang *et al.*, 2007; Lu *et al.*, 1998), as well as Tec (Kane and Watkins, 2005; Lachance *et al.*, 2002; Tomlinson *et al.*, 2004). TFKs have widely varying binding specificities and affinities for inositol compounds (Kojima *et al.*, 1997). The differences in the binding have been investigated by modeling the structures for Bmx, Itk, and Tec PH domains (Okoh and Vihinen, 1999).

A large set of phosphoinositide-binding PH domains in various species were identified by combining bioinformatics with experimental studies (Park *et al.*, 2008). This study confirmed the PIP<sub>3</sub>-inducible binding of Btk and Tec. Altogether, 40 PI3K-regulated PH domain-containing proteins were identified in vertebrates, four in *D. melanogaster*, one of which was Btk29A, but none in yeast. Amino acids across the whole PH domain were found to contribute to PIP<sub>3</sub> binding. The evolutionary interpretations from the study were that PIP<sub>3</sub> regulation of PH domains has evolved several times as independent events.

PI3K can be subdivided into three classes: IA, IB; II; and III (Vanhaesebroeck *et al.*, 1997). From an evolutionary point of view PI3K has been identified both in yeast and in plants (Herman *et al.*, 1992; Hong and Verma, 1994; Welters *et al.*, 1994). As PIP<sub>3</sub> can be generated via stimulation of many different receptors, including immunoreceptors, G protein-coupled receptors, as well as membrane-spanning tyrosine kinases, PI3K-induced activation of TFKs can be achieved through multiple pathways. At physiological concentrations, IP<sub>4</sub> enhances the binding of Itk's PH domain to PIP<sub>3</sub> (Huang *et al.*, 2007). In the case of Btk, its selective interaction with particular phosphoinositides has been addressed using biochemical and cell-biological methods (Hamman *et al.*, 2002; Nore *et al.*, 2000; Rameh *et al.*, 1997; Saito *et al.*, 2001; Salim *et al.*, 1996; Varnai *et al.*, 2005) as well as structure determination (Baraldi *et al.*, 1999).

### C. Regulation of TFKs through the PH domain by serine/threonine kinases

The PH domain also serves as an important region for the regulation of TFKs by serine/threonine kinases. However, the situation is quite complex with differential effects among family members. The activation of protein kinase C negatively regulates the activity of Btk (Yao *et al.*, 1994). These and other authors demonstrated that PKC $\beta$ 1 constitutively interacts with Btk *in vivo* and that both Ca<sup>2+</sup>-dependent and -independent forms of PKC could bind to Btk (Johannes *et al.*, 1999; Kawakami *et al.*, 2000; Yao *et al.*, 1994). Btk also serves as a substrate for



PKC and its enzymatic activity is downregulated by PKC-mediated phosphorylation. However, more recent studies have reported that the key regulatory site, S180, is in fact in the TH domain (Kang *et al.*, 2001; Venkataraman *et al.*, 2006).

In platelets, PKC $\alpha$  activates Btk, while Btk negatively regulates PKC $\alpha$  (Crosby and Poole, 2002). Although, PKC $\beta$ I and -II serve as negative regulators of Btk, the deletion of the gene encoding PKC $\beta$ I and -II in mice causes a phenocopy of Btk deficiency (Leitges *et al.*, 1996). In Itk, the situation may be different, since PKC seems to activate it (Kawakami *et al.*, 1996).

Tec binds constitutively to PKC $\theta$  through its PH domain (Altman *et al.*, 2004). The Bmx-induced DNA binding of Stat1 is selectively inhibited by PKC $\delta$ . The coexpression of Bmx with PKC $\delta$ -induced phosphorylation of this isoform of PKC (Saharinen *et al.*, 1997). The interaction between PH domains and PKC seems to extend to at least some other PH domains, since the PH domain of G protein-coupled receptor kinase-2, GRK2, binds to PKC and affects the activity of this kinase (Yang *et al.*, 2003).

Evolutionarily, PKCs are ancient and found also in plants (Bögre *et al.*, 2003; Zegzouti *et al.*, 2006) and in yeast (Levin *et al.*, 1990). The same is true for phospholipase C, which appeared more than 1000 million years ago (Hirayama *et al.*, 1995; Koyanagi *et al.*, 1998; Tasma *et al.*, 2008). PLC $\gamma$ 2 acts upstream of PKCs and serves as a substrate for Btk, which phosphorylates two tyrosines in a linker between the SH2 and SH3 domains (Humphries *et al.*, 2004; Kim *et al.*, 2004).

Recently, the importance of serines and PSK phosphorylation sites has been revealed from another direction. Peptidylprolyl-isomerase Pin1 targets in BTK two PH domain dipeptides, S21–P22 and S115–P116, and acts as a negative regulator. Pin1 affects the most N-terminal site in mitosis and S115–P116 during the interphase (Yu *et al.*, 2006). Corresponding serine/threonine kinases or phosphatases have not been identified until now. Pin1 appears also in plants (Yao *et al.*, 2001) and in yeast (Behrsin *et al.*, 2007; Hanes *et al.*, 1989; Lu *et al.*, 1996).

## D. Function of the TH domain

Another conserved, characteristic region of TFKs is the TH domain, and especially its N-terminal Btk motif (Smith *et al.*, 1994b; Vihinen *et al.*, 1994a, 1997a). While the PRR differs among TFKs, the Btk motif is highly conserved. Its only known function is to stabilize the PH domain through the conformational interaction with a Zn<sup>2+</sup> ion, as demonstrated for Btk (Baraldi *et al.*, 1999; Hyvönen and Saraste, 1997). The entire TH domain is unique for TFKs. The Btk motif appears also in some other proteins. In the in-depth report of TH domain identification (Vihinen *et al.*, 1994a) an unidentified partial protein was detected, which turned out to be Ras GTPase-activating protein (GAP) (Vihinen *et al.*, 1997a).

As discussed above, the PRR part of the TH domain can interact with the SH3 domain in an inter- or intramolecular manner. Whether additional functions exist is not known. Missense mutations affecting patients with XLA result in a very unstable protein, which readily is degraded (Vihinen *et al.*, 1997a). Structure for separately expressed domains in Bmx and Itk with Zn<sup>2+</sup> have been solved.

## E. Regulation of Btk through SH3BP5

SH3BP5 (Sab) was originally identified in mammals as a novel adaptor protein, which binds to the SH3 domain of Btk with high preference (Matsushita *et al.*, 1998). SH3BP5 also controls negatively B-cell antigen-mediated signaling (Yamadori *et al.*, 1999). *D. melanogaster* SH3BP5 (Sinka *et al.*, 2002), also denoted Parcas, was demonstrated in a genetic screen to act as a negative regulator of Btk29A (Hamada *et al.*, 2005).

SH3BP5 also interacts with c-Jun N-terminal kinase (JNK) (Wiltshire *et al.*, 2002). As with c-Jun, the JNK interaction is mediated through its putative mitogen-activated protein kinase interaction motifs (KIMs) (Wiltshire *et al.*, 2002, 2004). Active JNK and phosphorylated Sab are colocalized and associated with mitochondria (Wiltshire *et al.*, 2002). These findings suggest a role in crosstalk between Btk and JNK signaling pathways (Wiltshire *et al.*, 2002, 2004).

A SH3BP5-like (SH3BP5L) protein shares sequence similarity with SH3BP5 (Strausberg *et al.*, 2002). No biological function has been characterized for it yet.

## F. Mutations in X-linked agammaglobulinemia as a tool to study the function of residues

XLA, being the protein kinase with the largest number of disease-related mutation among all human protein kinases (Ortutay *et al.*, 2005), has allowed us and others to investigate the significance and structure–function relationships of several regions and amino acids in TFKs and other kinases. Missense and other in-frame mutations have been instrumental in these studies. Due to the conservation of TFKs along the entire sequence it is relatively easy to interpolate information for one family to others as in the case of JAK3 (Notarangelo *et al.*, 2001; Vihinen *et al.*, 2000). The importance of such data is not limited to TFKs, since when the naturally occurring, disease-causing mutation R28C in the Btk PH domain, in both in man and mice, was introduced to the Akt kinase, a stable but inactive protein was obtained (Lehnes *et al.*, 2007; Sable *et al.*, 1998; Stoica *et al.*, 2003).

Since 1995 mutations in the *BTK* gene have been collected. The BTKbase (<http://bioinf.uta.fi/BTKbase>) database is freely available and has served as a model for some 130 additional mutation databases, mainly for

immunodeficiencies (Piiirilä *et al.*, 2006). BTKbase has constantly grown from 188 cases to the current number of 1096 patients (Lindvall *et al.*, 2005; Väliäho *et al.*, 2006; Vihinen *et al.*, 1995a,b, 1996, 1997b, 1998, 1999, 2001). Experimental and modeled structures for BTK domains have extensively been used to explain protein structure–function relationships and consequences of mutations (Holinski-Feder *et al.*, 1998; Jin *et al.*, 1995; Korpi *et al.*, 2000; Lindvall *et al.*, 2005; Maniar *et al.*, 1995; Mao *et al.*, 2001; Mattsson *et al.*, 2000; Okoh *et al.*, 2002; Speletas *et al.*, 2001; Väliäho *et al.*, 2006; Vihinen *et al.*, 1994b,c, 1995b; Vorechovsky *et al.*, 1995, 1997; Zhu *et al.*, 1994). These studies have allowed us to provide putative functional and/or structural explanation for all XLA-causing mutations. The quality of the structural predictions was retrospectively assessed and found to be very good (Khan and Vihinen, submitted for publication).

Mutations have also been detected in the BTK promoter region affecting a highly conserved binding site for Ets family transcription factors (Holinski-Feder *et al.*, 1998). Btk has been reported to activate NF- $\kappa$ B signaling (Bajpai *et al.*, 2000; Petro *et al.*, 2000). Recently Btk was shown to autoregulate its promoter in a positive fashion (Yu *et al.*, 2008). Interestingly, also Bmx, Itk, and Tec seem to be positively regulated by NF- $\kappa$ B (Yu *et al.*, 2008, manuscript in preparation).

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## IV. THE ANCESTRY OF TFKs

### A. Collecting the sequences

Multiple blastp (Altschul *et al.*, 1997) searches were made in an iterative manner against GenBank nonredundant protein database to identify TFK sequences. In the first step, human TFK protein sequences were used as query and the results were examined by distant tree representation with the online tool provided by the National Center for Biotechnology Information (NCBI). All the detected sequences were carefully checked and only full-length entries were accepted. When sequence variants appeared, they were aligned to the query sequence and the one closest to the query with the longest sequence was selected. From each search only those sequences, which were closest to the human query were included to the result set, that is, only the orthologs were included. Sequences for which the orthology was not so clear were collected separately. Additional search was made by using the fruit fly Btk29A sequence as the query.

TFK members were identified also from some incomplete genome datasets. Unannotated TFK sequences were searched from *Xenopus laevis* (Bowes *et al.*, 2008), *Xenopus tropicalis* (Bowes *et al.*, 2008), *Takifugu rubripes* (Aparicio *et al.*, 2002), and *Danio rerio* (zebrafish) (Sprague *et al.*, 2008) genomes. Several tblastn (Altschul *et al.*, 1997) searches were performed using TFK protein

sequences from *Gallus gallus* (chicken) as queries. An obviously missing Btk sequence from *Macaca mulatta* was identified by using a tblastn search against Macaca mRNA database (Gibbs *et al.*, 2007). A likely mistranslated mRNA was detected with a frameshift caused by a possible sequencing error. The sequence was reconstructed by translating the mRNA in six reading frames and by aligning the protein sequences with the human BTK protein sequence. Partial sequences, such as those for Tec and Txk in *Sus scrofa* (wild boar), were excluded.

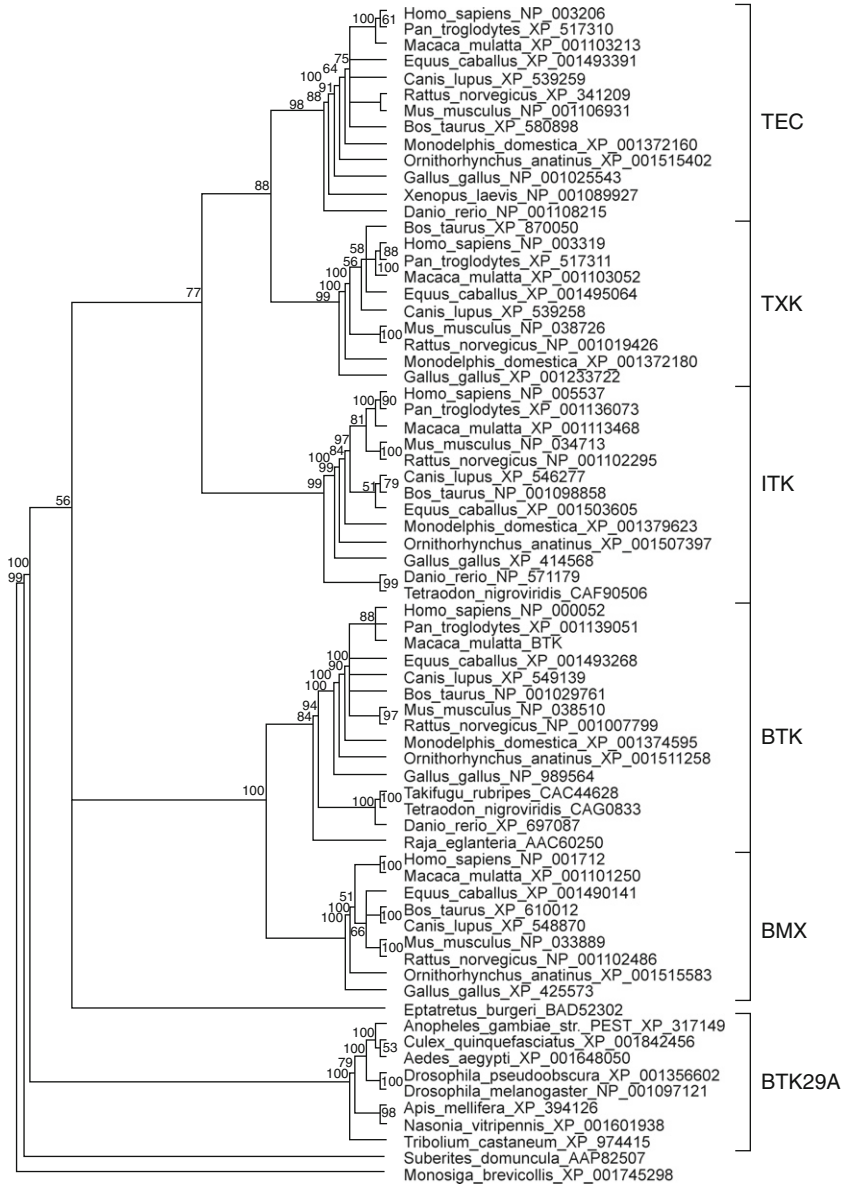
## B. Aligning the sequences

The identified TFK sequences were divided into seven groups: the chordate-specific Bmx, Btk, Itk, Tec, and Txk groups and the insect-specific Btk29A-related sequences. The seventh group is for three sequences equally distant from all the others. This outgroup contained sequences BAD52302 from *Eptatretus burgeri* (hagfish), XP\_001745298 from *Monosiga brevicollis* (choanoflagellate), and AAP82507 from *Suberites domuncula* (sponge). The six protein groups were aligned individually and finally combined with the outgroup sequences. In this way the internal conservation of the protein groups was better preserved in the final alignment.

## C. Phylogenetic analysis

Based on the multiple sequence alignment, a bootstrap analysis was performed using maximum parsimony as criteria for searching the optimal tree (Fig. 3.1). The six protein groups are clearly separated on the tree, and we can draw the phylogeny of these proteins as follows. The ancestor of all the TFKs was present in early eukaryotes prior to the formation of metazoans. The sequences from *S. domuncula* and *M. brevicollis* are orthologs of the ancestor. After the divergence of deuterostomia and protostomia the descendants of the ancestor further diverged. In protostomia, now insects, TFKs developed in the form of the Btk29A protein group.

In deuterostomia a descendant of the single gene became the ancestor for the five chordata-specific protein groups. The TFK in *E. burgeri* is a direct ascendant to that. After the formation of craniata, but before the formation of vertebrata, the ancestor went through multiple duplications. First, it was divided into the Btk/Bmx and Tec/Txk/Itk groups. Then both groups duplicated until all the five protein groups appeared. These events took place before the emergence of vertebrates. The lack of sequences in fishes and some other genomes is likely due to deletion events rather than duplications after the emergence of the vertebrates, since all the sequences within the groups are more similar to each other than to any of the fish or frog sequences.



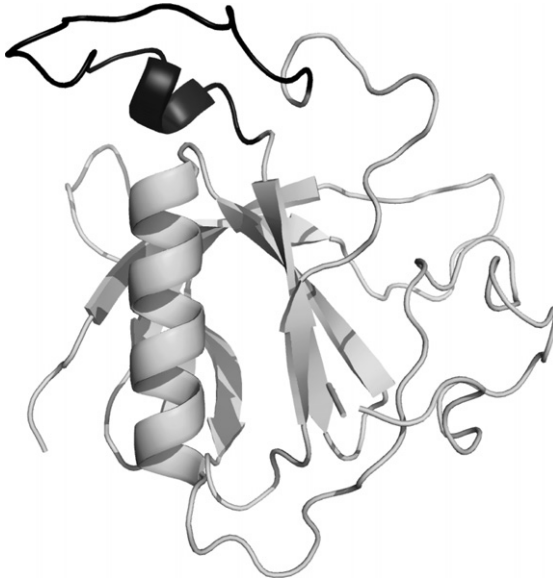
**Figure 3.1.** Phylogenetic relationship of the TFK sequences. The six major protein groups can be clearly distinguished. Bootstrap analysis was carried out using maximum parsimony criteria and 100 replicates with PAUP\* (Swofford, 2003). Bootstrap values are shown at the nodes. Sequence labels contain name of the species and NCBI Entrez accession number for the protein sequence. Btk sequence for *Macaca mulatta* was reconstructed by transcribing the mRNA record XR\_011285.1 and correcting the disrupted frameshift.

Another view on evolution is based upon the analysis of genomic organization. Recently the amphioxus, *Branchiostoma floridae*, genome was published (Putnam *et al.*, 2008). The authors partly reconstructed the genomic organization of the last common chordate ancestor and described two genome-wide duplications and subsequent reorganizations in the vertebrate lineage. Interestingly, number 8 of the 17 reconstructed ancestral chordate linkage groups contains regions corresponding to the location of all TFKs in the human genome.

Since we identified only a single frog TFK, the genomic region was analyzed. According to Xenbase (<http://www.xenbase.org/>), *Cyfp2* and *Med7* genes are both located in the vicinity of the *Tec* gene. This is surprising since both these genes are in close proximity to the *Itk* gene in zebrafish, mouse and man. The *Itk* and *Tec* genes in these three species are on different chromosomes. The *Txk* gene, which is absent from the zebrafish, is in very close proximity to the *Tec* gene in humans and mice. However, there is no doubt from the sequence alignment that the frog TFK should be classified as *Tec*. It is possible that a recombination event in an ancestor have transferred the *Tec* gene from its original location into the position of an *Itk* homolog, which was simultaneously lost. An alternative explanation is that the gene has evolved so that it is currently more closer to *Tec* than to *Itk*. The *X. laevis* *Tec* has similarity to *Tec* from other species throughout the sequence, suggesting that if a recombination occurred, the entire gene was replaced.

#### **D. The origin of the Btk-specific PH domain loop in amniotes**

Many Btk sequences contain a characteristic loop of 20 residues between amino acids 78 and 98 (numbering according to BTK). It is present only in the mammalian and bird Btk orthologs, not in the other TFKs. Exon 4 in these genes encodes residues 81–103 (human BTK numbering). We assume that the loop emerged by the insertion of exon 4 into the *Btk* gene. This insertion was tolerated, because the loop is on the surface of the protein (Fig. 3.2). The termini are close to each other in the three-dimensional space as in many protein domains, which appear in several proteins surrounded by different structural regions. In the loop region, there are in BTKbase, five frameshift mutations, which lead to premature stop codon and truncated product, three nonsense mutations, and a duplication, which causes a frameshift and premature termination. These mutations are disease causing, because they produce a nonfunctional, truncated protein. There is just one missense mutation, F98V substitution. The presence of a single, disease-causing missense mutation is not very informative, since this stretch is rather short and does not contain any hotspots for mutations, such as repetitive sequences or CpG dinucleotides. The loop is highly conserved.



**Figure 3.2.** Amniotes specific insert in the PH domain of BTK. The insert is protruding up as a dark loop in the 1BTK structure containing the PH domain and BTK motif from human BTK. The loop is mainly nonstructured, but contains a short  $\alpha$ -helix.

There are just minor variations in the sequence alignment, which suggests that selection pressure has maintained the sequence. Whether the underlying mechanism is functional or has a different origin is not clear.

There is also a missense SNP in the region, rs56035945 with R82K alteration, without known phenotype. Chicken, platypus and opossum Btk's have a lysine at that site while there is arginine in the higher mammals. Arginine and lysine are both basic residues and frequently substituted by each other in protein families.

### **E. N-terminal regions of insect TFKs**

*D. melanogaster* has three alternatively spliced mRNA forms, whereas a single form appears in other insects. The three mRNA variants code for two alternative protein products, because two of them differ just in their 5'-untranslated regions. The difference in the variants is that the longer form encompasses exons 1–5 and 9–16, while the shorter form contains exons 5–16. The longer protein variant aligns with that from *D. pseudoobscura*, while the shorter one with the proteins from *Anopheles gambiae* and *Culex pipiens quinquefasciatus*. Also other dipteran genomes might have alternatively spliced forms.

The N-terminus in insect proteins does not align at all with other TFK proteins before the beginning of the SH3 domain from whereon they align very well. Since this region is not conserved at all, and the whole N-terminus is truncated in the *Aedes aegypti* sequence, its specific function remains elusive.

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## **V. SH3BP5—A CONSERVED NEGATIVE REGULATOR OF TFKs**

The SH3-binding protein 5 interacts with Btk and MAP kinases. The ortholog sequences of human SH3BP5 protein were collected in a similar way as for TFKs. As there were closely related paralogs called SH3BP5L in several genomes they were included to the analysis.

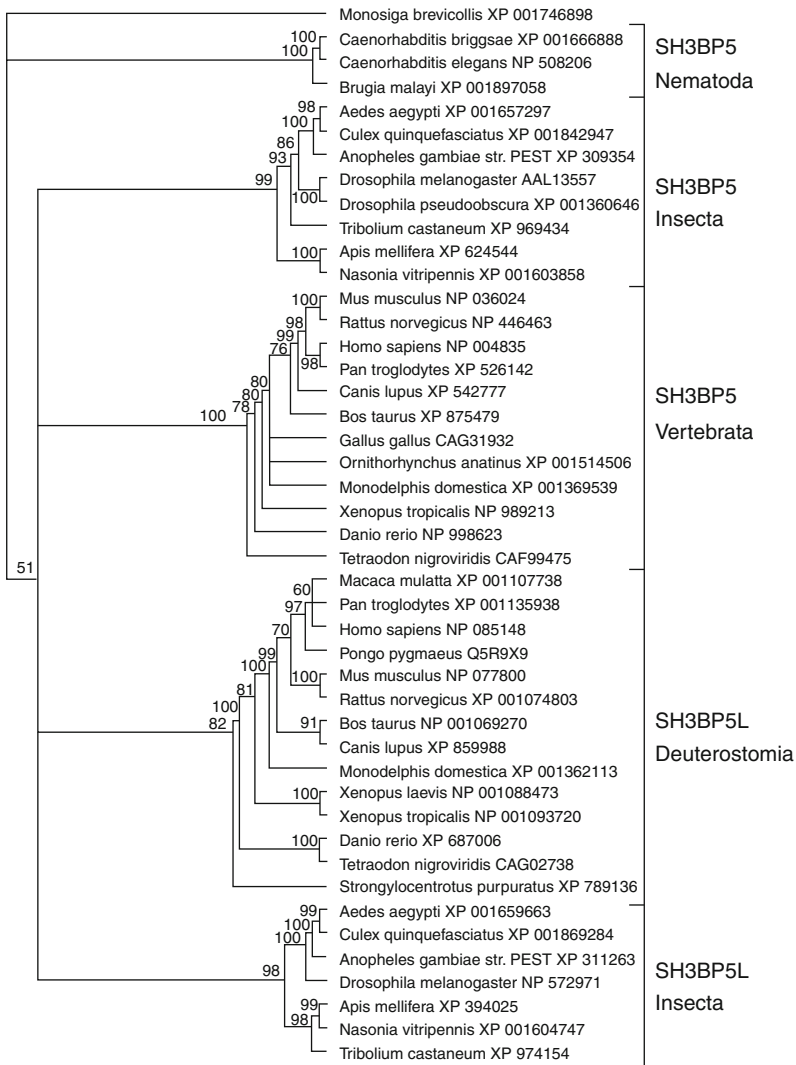
In the tree, there are several protein groups (Fig. 3.3). An ortholog appears also in *M. brevicollis*, but does not fit to any of the groups. The insect and vertebrate groups for both SH3BP5 and SH3BP5L can be clearly distinguished along with a group in nematodes. The relationship of these big groups is more difficult to resolve. The simplest explanation for the tree is that there was a single SH3BP5 gene in the ancestors of eukaryotes and a duplication appeared very early. Both the duplicons were then preserved in insect as well as vertebrate lineages. The nematode group does not fit easily to this explanation. The role of both SH3BP5 and SH3BP5L proteins has to be significant, since they can be identified from all the important branches of eukaryotes. The detailed function of these proteins remains to be elucidated.

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## **VI. THE ORIGIN OF PHOSPHOTYROSINE SIGNALING AND THE ROLE OF CYTOPLASMIC TYROSINE KINASES**

The evolution of phosphotyrosine signaling suggests that more than 600 million years ago there was a common ancestor for the unicellular choanoflagellates and for multicellular metazoans, which had already developed this ability (King and Carroll, 2001; King *et al.*, 2008; Peterson and Butterfield, 2005; Pincus *et al.*, 2008). In some species, such as in yeast, tyrosine phosphorylation appears at a very low level, most likely due to promiscuity of serine/threonine kinases (Schieven *et al.*, 1986). In a recent report, Pincus *et al.* (2008) suggest that phosphatases and SH2 domains appeared first, whereas the enzymatic activity of tyrosine kinases developed later. The emergence of specific proteins resulted in the expansion of proteins and domains in cellular signaling. One third of all domains found in combination with SH2 domains in choanoflagellates are unique while 38% are shared with metazoans.





**Figure 3.3.** Phylogenetic relationship of the SH3-binding protein 5 and related sequences. Groups of SH3BP5 and SH3BP5L proteins from vertebrates and insects can be distinguished in addition to a distinct group of SH3BP5 proteins from nematodes. Further relationship of these groups is not clear. The analysis was carried out as described in Fig. 3.1.

Proteins active in tyrosine kinase-related signaling are quite abundant in choanoflagellates (King *et al.*, 2008). While most proteins in serine–threonine signaling pathways are common between metazoans and choanoflagellates, the opposite is true for tyrosine phosphorylation and several other intracellular signaling pathways, including many transcription factors.

Among choanoflagellates, SFKs and C-terminal Src kinase (Csk) were first reported in *M. ovata* (Segawa *et al.*, 2006). The *M. ovata* Src has transforming capability but is not negatively regulated by Csk. Biochemical characterization of the *M. brevicollis* Src and Csk indicated that the putative, regulatory C-terminal tyrosine is not phosphorylated (Li *et al.*, 2008).

Our report is the first to demonstrate the existence of a TFK in *M. brevicollis*, which has estimated to have 128 tyrosine kinase genes (King *et al.*, 2008). Although the role of the *M. brevicollis* TFK is unknown, its mere existence clearly suggests that it is functionally active in a unicellular organism. Since all domains of TFKs are conserved in this protein, it is possible that this kinase is already regulated by SFKs, PI3K, and PKC, like the metazoan counterparts. However, given the lack of Csk-induced control of SFKs in *M. brevicollis* as well as in *M. ovata*, it is equally possible that the regulation differs. Choanoflagellates also encode an SH3BP5-related molecule, which has not been functionally characterized. Thus, it is too early to say whether this molecule suppresses the corresponding TFK. Functional studies will be needed to resolve this issue as well as the possibility that the choanoflagellate TFK can substitute for the loss of TFKs in metazoan cells.

Our study of TFKs reveals that these enzymes are ancient and their ancestor appeared already in choanoflagellates. TFK members are regulated by several proteins and they control numerous signaling pathways. More studies will be needed to investigate how the pathways in which TFKs currently participate originally obtained this property.

## Acknowledgments

This work was supported by The Swedish Cancer Fund, The Wallenberg Foundation, the Swedish Science Council, the Stockholm County Council (research grant ALF-projektmedel), the European Union grant FP7-HEALTH-F2-2008-201549, the Medical Research Fund of Tampere University Hospital, and Academy of Finland.

## References

- Altman, A., Kaminski, S., Busuttill, V., Droin, N., Hu, J., Tadevosyan, Y., Hipskind, R. A., and Villalba, M. (2004). Positive feedback regulation of PLC $\gamma$ 1/Ca<sup>(2+)</sup> signaling by PKC $\theta$  in restimulated T cells via a Tec kinase-dependent pathway. *Eur. J. Immunol.* **34**, 2001–2011.
- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389–3402.
- Andreotti, A. H., Bunnell, S. C., Feng, S., Berg, L. J., and Schreiber, S. L. (1997). Regulatory intramolecular association in a tyrosine kinase of the Tec family. *Nature* **385**, 93–97.
- Aparicio, S., Chapman, J., Stupka, E., Putnam, N., Chia, J. M., Dehal, P., Christoffels, A., Rash, S., Hoon, S., Smit, A., Gelpke, M. D., Roach, J., *et al.* (2002). Whole-genome shotgun assembly and analysis of the genome of *Fugu rubripes*. *Science* **297**, 1301–1310.

- Atkinson, B. T., Ellmeier, W., and Watson, S. P. (2003). Tec regulates platelet activation by GPVI in the absence of Btk. *Blood* **102**, 3592–3599.
- August, A., Sadra, A., Dupont, B., and Hanafusa, H. (1997). Src-induced activation of inducible T cell kinase (ITK) requires phosphatidylinositol 3-kinase activity and the pleckstrin homology domain of inducible T cell kinase. *Proc. Natl Acad. Sci. USA* **94**, 11227–11232.
- Baba, K., Takeshita, A., Majima, K., Ueda, R., Kondo, S., Juni, N., and Yamamoto, D. (1999). The *Drosophila* Bruton's tyrosine kinase (Btk) homolog is required for adult survival and male genital formation. *Mol. Cell. Biol.* **19**, 4405–4413.
- Bäckesjö, C. M., Vargas, L., Superti-Furga, G., and Smith, C. I. E. (2002). Phosphorylation of Bruton's tyrosine kinase by *c*-Abl. *Biochem. Biophys. Res. Commun.* **299**, 510–515.
- Bajpai, U. D., Zhang, K., Teutsch, M., Sen, R., and Wortis, H. H. (2000). Bruton's tyrosine kinase links the B cell receptor to nuclear factor  $\kappa$ B activation. *J. Exp. Med.* **191**, 1735–1744.
- Baraldi, E., Djinic Carugo, K., Hyvönen, M., Surdo, P. L., Riley, A. M., Potter, B. V., O'Brien, R., Ladbury, J. E., and Saraste, M. (1999). Structure of the PH domain from Bruton's tyrosine kinase in complex with inositol 1,3,4,5-tetrakisphosphate. *Structure* **7**, 449–460.
- Behrsin, C. D., Bailey, M. L., Bateman, K. S., Hamilton, K. S., Wahl, L. M., Brandl, C. J., Shilton, B. H., and Litchfield, D. W. (2007). Functionally important residues in the peptidylprolyl isomerase Pin1 revealed by unigenic evolution. *J. Mol. Biol.* **365**, 1143–1162.
- Berg, L. J., Finkelstein, L. D., Lucas, J. A., and Schwartzberg, P. L. (2005). Tec family kinases in T lymphocyte development and function. *Annu. Rev. Immunol.* **23**, 549–600.
- Bögre, L., Okresz, L., Henriques, R., and Anthony, R. G. (2003). Growth signalling pathways in Arabidopsis and the AGC protein kinases. *Trends Plant Sci.* **8**, 424–431.
- Bowes, J. B., Snyder, K. A., Segerdell, E., Gibb, R., Jarabek, C., Noumen, E., Pollet, N., and Vize, P. D. (2008). Xenbase: A *Xenopus* biology and genomics resource. *Nucleic Acids Res.* **36**, D761–D767.
- Brazin, K. N., Fulton, D. B., and Andreotti, A. H. (2000). A specific intermolecular association between the regulatory domains of a Tec family kinase. *J. Mol. Biol.* **302**, 607–623.
- Breheny, P. J., Laederach, A., Fulton, D. B., and Andreotti, A. H. (2003). Ligand specificity modulated by prolyl imide bond *cis/trans* isomerization in the Itk SH2 domain: A quantitative NMR study. *J. Am. Chem. Soc.* **125**, 15706–15707.
- Broussard, C., Fleischacker, C., Horai, R., Chetana, M., Venegas, A. M., Sharp, L. L., Hedrick, S. M., Fowlkes, B. J., and Schwartzberg, P. L. (2006). Altered development of CD8+ T cell lineages in mice deficient for the Tec kinases Itk and Rlk. *Immunity* **25**, 93–104.
- Brown, K., Long, J. M., Vial, S. C., Dedi, N., Dunster, N. J., Renwick, S. B., Tanner, A. J., Frantz, J. D., Fleming, M. A., and Cheetham, G. M. (2004). Crystal structures of interleukin-2 tyrosine kinase and their implications for the design of selective inhibitors. *J. Biol. Chem.* **279**, 18727–18732.
- Brunner, C., Muller, B., and Wirth, T. (2005). Bruton's tyrosine kinase is involved in innate and adaptive immunity. *Histol. Histopathol.* **20**, 945–955.
- Caenepeel, S., Charyczak, G., Sudarsanam, S., Hunter, T., and Manning, G. (2004). The mouse kinome: Discovery and comparative genomics of all mouse protein kinases. *Proc. Natl Acad. Sci. USA* **101**, 11707–11712.
- Cetkovic, H., Muller, W. E., and Gamulin, V. (2004). Bruton tyrosine kinase-like protein, *btksd*, is present in the marine sponge *Suberites domuncula*. *Genomics* **83**, 743–745.
- Chandrasekaran, V., and Beckendorf, S. K. (2005). Tec29 controls actin remodeling and endoreplication during invagination of the *Drosophila* embryonic salivary glands. *Development* **132**, 3515–3524.
- Crosby, D., and Poole, A. W. (2002). Interaction of Bruton's tyrosine kinase and protein kinase *C* $\theta$  in platelets. Cross-talk between tyrosine and serine/threonine kinases. *J. Biol. Chem.* **277**, 9958–9965.

- Ekman, N., Lymboussaki, A., Västriik, I., Sarvas, K., Kaipainen, A., and Alitalo, K. (1997). Bmx tyrosine kinase is specifically expressed in the endocardium and the endothelium of large arteries. *Circulation* **96**, 1729–1732.
- Ekman, N., Arighi, E., Rajantie, I., Saharinen, P., Riskimäki, A., Silvennoinen, O., and Alitalo, K. (2000). The Bmx tyrosine kinase is activated by IL-3 and G-CSF in a PI-3K dependent manner. *Oncogene* **19**, 4151–4158.
- Ellmeier, W., Jung, S., Sunshine, M. J., Hatam, F., Xu, Y., Baltimore, D., Mano, H., and Littman, D. R. (2000). Severe B cell deficiency in mice lacking the tec kinase family members Tec and Btk. *J. Exp. Med.* **192**, 1611–1624.
- Felices, M., and Berg, L. J. (2008). The Tec kinases Itk and Rlk regulate NKT cell maturation, cytokine production, and survival. *J. Immunol.* **180**, 3007–3018.
- Felices, M., Falk, M., Kosaka, Y., and Berg, L. J. (2007). Tec kinases in T cell and mast cell signaling. *Adv. Immunol.* **93**, 145–184.
- Finkelstein, L. D., and Schwartzberg, P. L. (2004). Tec kinases: Shaping T-cell activation through actin. *Trends Cell Biol.* **14**, 443–451.
- Gibbs, R. A., Rogers, J., Katze, M. G., Bumgarner, R., Weinstock, G. M., Mardis, E. R., Remington, K. A., Strausberg, R. L., Venter, J. C., Wilson, R. K., Batzer, M. A., Bustamante, C. D., *et al.* (2007). Evolutionary and biomedical insights from the rhesus macaque genome. *Science* **316**, 222–234.
- Gomez-Rodriguez, J., Readinger, J. A., Viorritto, I. C., Mueller, K. L., Houghtling, R. A., and Schwartzberg, P. L. (2007). Tec kinases, actin, and cell adhesion. *Immunol. Rev.* **218**, 45–64.
- Guarnieri, D. J., Dodson, G. S., and Simon, M. A. (1998). SRC64 regulates the localization of a Tec-family kinase required for *Drosophila* ring canal growth. *Mol. Cell* **1**, 831–840.
- Haire, R. N., Ohta, Y., Lewis, J. E., Fu, S. M., Kroisel, P., and Litman, G. W. (1994). TXK, a novel human tyrosine kinase expressed in T cells shares sequence identity with Tec family kinases and maps to 4p12. *Hum. Mol. Genet.* **3**, 897–901.
- Haire, R. N., Strong, S. J., and Litman, G. W. (1997). Identification and characterization of a homologue of Bruton's tyrosine kinase, a Tec kinase involved in B-cell development, in a modern representative of a phylogenetically ancient vertebrate. *Immunogenetics* **46**, 349–351.
- Haire, R. N., Strong, S. J., and Litman, G. W. (1998). Tec-family non-receptor tyrosine kinase expressed in zebrafish kidney. *Immunogenetics* **47**, 336–337.
- Hamada, N., Bäckesjö, C. M., Smith, C. I. E., and Yamamoto, D. (2005). Functional replacement of *Drosophila* Brk29A with human Btk in male genital development and survival. *FEBS Lett.* **579**, 4131–4137.
- Hamman, B. D., Pollok, B. A., Bennett, T., Allen, J., and Heim, R. (2002). Binding of a pleckstrin homology domain protein to phosphoinositide in membranes: A miniaturized FRET-based assay for drug screening. *J. Biomol. Screen.* **7**, 45–55.
- Hanes, S. D., Shank, P. R., and Bostian, K. A. (1989). Sequence and mutational analysis of ESS1, a gene essential for growth in *Saccharomyces cerevisiae*. *Yeast* **5**, 55–72.
- Hansson, H., Mattsson, P. T., Allard, P., Haapaniemi, P., Vihinen, M., Smith, C. I. E., and Härd, T. (1998). Solution structure of the SH3 domain from Bruton's tyrosine kinase. *Biochemistry* **37**, 2912–2924.
- Hansson, H., Okoh, M. P., Smith, C. I. E., Vihinen, M., and Härd, T. (2001a). Intermolecular interactions between the SH3 domain and the proline-rich TH region of Bruton's tyrosine kinase. *FEBS Lett.* **489**, 67–70.
- Hansson, H., Smith, C. I. E., and Härd, T. (2001b). Both proline-rich sequences in the TH region of Bruton's tyrosine kinase stabilize intermolecular interactions with the SH3 domain. *FEBS Lett.* **508**, 11–15.
- Hao, S., and August, A. (2002). The proline rich region of the Tec homology domain of ITK regulates its activity. *FEBS Lett.* **525**, 53–58.

- Hasan, M., Lopez-Herrera, G., Blomberg, K. E., Lindvall, J. M., Berglöf, A., Smith, C. I. E., and Vargas, L. (2008). Defective Toll-like receptor 9-mediated cytokine production in B cells from Bruton's tyrosine kinase-deficient mice. *Immunology* **123**, 239–249.
- He, Y., Luo, Y., Tang, S., Rajantie, I., Salven, P., Heil, M., Zhang, R., Luo, D., Li, X., Chi, H., Yu, J., Carmeliet, P., *et al.* (2006). Critical function of Bmx/Etk in ischemia-mediated arteriogenesis and angiogenesis. *J. Clin. Invest.* **116**, 2344–2355.
- Herman, P. K., Stack, J. H., and Emr, S. D. (1992). An essential role for a protein and lipid kinase complex in secretory protein sorting. *Trends Cell Biol.* **2**, 363–368.
- Heyeck, S. D., and Berg, L. J. (1993). Developmental regulation of a murine T-cell-specific tyrosine kinase gene, *Tsk*. *Proc. Natl Acad. Sci. USA* **90**, 669–673.
- Hirayama, T., Ohto, C., Mizoguchi, T., and Shinozaki, K. (1995). A gene encoding a phosphatidylinositol-specific phospholipase C is induced by dehydration and salt stress in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* **92**, 3903–3907.
- Holinski-Feder, E., Weiss, M., Brandau, O., Jedele, K. B., Nore, B., Bäckesjö, C. M., Vihinen, M., Hubbard, S. R., Belohradsky, B. H., Smith, C. I. E., and Meindl, A. (1998). Mutation screening of the *BTK* gene in 56 families with X-linked agammaglobulinemia (XLA): 47 unique mutations without correlation to clinical course. *Pediatrics* **101**, 276–284.
- Hong, Z., and Verma, D. P. (1994). A phosphatidylinositol 3-kinase is induced during soybean nodule organogenesis and is associated with membrane proliferation. *Proc. Natl Acad. Sci. USA* **91**, 9617–9621.
- Hu, Q., Davidson, D., Schwartzberg, P. L., Macchiarini, F., Lenardo, M. J., Bluestone, J. A., and Matis, L. A. (1995). Identification of Rlk, a novel protein tyrosine kinase with predominant expression in the T cell lineage. *J. Biol. Chem.* **270**, 1928–1934.
- Huang, K. C., Cheng, H. T., Pai, M. T., Tzeng, S. R., and Cheng, J. W. (2006). Solution structure and phosphopeptide binding of the SH2 domain from the human Bruton's tyrosine kinase. *J. Biomol. NMR* **36**, 73–78.
- Huang, Y. H., Grasis, J. A., Miller, A. T., Xu, R., Soonthornvacharin, S., Andreotti, A. H., Tsoukas, C. D., Cooke, M. P., and Sauer, K. (2007). Positive regulation of Itk PH domain function by soluble IP<sub>4</sub>. *Science* **316**, 886–889.
- Humphries, L. A., Dangelmaier, C., Sommer, K., Kipp, K., Kato, R. M., Griffith, N., Bakman, I., Turk, C. W., Daniel, J. L., and Rawlings, D. J. (2004). Tec kinases mediate sustained calcium influx via site-specific tyrosine phosphorylation of the phospholipase C $\gamma$  Src homology 2-Src homology 3 linker. *J. Biol. Chem.* **279**, 37651–37661.
- Hyvönen, M., and Saraste, M. (1997). Structure of the PH domain and Btk motif from Bruton's tyrosine kinase: Molecular explanations for X-linked agammaglobulinaemia. *EMBO J.* **16**, 3396–3404.
- Ingle, E. (2008). Src family kinases: Regulation of their activities, levels and identification of new pathways. *Biochim. Biophys. Acta* **1784**, 56–65.
- Islam, T. C., and Smith, C. I. E. (2000). The cellular phenotype conditions Btk for cell survival or apoptosis signaling. *Immunol. Rev.* **178**, 49–63.
- Jiang, X., Borgesi, R. A., McKnight, N. C., Kaur, R., Carpenter, C. L., and Balk, S. P. (2007). Activation of nonreceptor tyrosine kinase Bmx/Etk mediated by phosphoinositide 3-kinase, epidermal growth factor receptor, and *erbB3* in prostate cancer cells. *J. Biol. Chem.* **282**, 32689–32698.
- Jin, H., Webster, A. D., Vihinen, M., Sideras, P., Vorechovsky, I., Hammarström, L., Bernatowska-Matuszkiewicz, E., Smith, C. I. E., Bobrow, M., and Vetrie, D. (1995). Identification of Btk mutations in 20 unrelated patients with X-linked agammaglobulinaemia (XLA). *Hum. Mol. Genet.* **4**, 693–700.
- Johannes, F. J., Hausser, A., Storz, P., Truckenmüller, L., Link, G., Kawakami, T., and Pfizenmaier, K. (1999). Bruton's tyrosine kinase (Btk) associates with protein kinase C  $\mu$ . *FEBS Lett.* **461**, 68–72.

- Jongstra-Bilen, J., Puig Cano, A., Hasija, M., Xiao, H., Smith, C. I. E., and Cybulsky, M. I. (2008). Dual functions of Bruton's tyrosine kinase and Tec kinase during Fc $\gamma$  receptor-induced signaling and phagocytosis. *J. Immunol.* **181**, 288–298.
- Joseph, R. E., Fulton, D. B., and Andreotti, A. H. (2007a). Mechanism and functional significance of Itk autophosphorylation. *J. Mol. Biol.* **373**, 1281–1292.
- Joseph, R. E., Min, L., and Andreotti, A. H. (2007b). The linker between SH2 and kinase domains positively regulates catalysis of the Tec family kinases. *Biochemistry* **46**, 5455–5462.
- Joseph, R. E., Min, L., Xu, R., Musselman, E. D., and Andreotti, A. H. (2007c). A remote substrate docking mechanism for the Tec family tyrosine kinases. *Biochemistry* **46**, 5595–5603.
- Kane, L. P., and Watkins, S. C. (2005). Dynamic regulation of Tec kinase localization in membrane-proximal vesicles of a T cell clone revealed by total internal reflection fluorescence and confocal microscopy. *J. Biol. Chem.* **280**, 21949–21954.
- Kang, S. W., Wahl, M. I., Chu, J., Kitaura, J., Kawakami, Y., Kato, R. M., Tabuchi, R., Tarakhovskiy, A., Kawakami, T., Turck, C. W., Witte, O. N., and Rawlings, D. J. (2001). PKC $\beta$  modulates antigen receptor signaling via regulation of Btk membrane localization. *EMBO J.* **20**, 5692–5702.
- Kawakami, Y., Yao, L., Han, W., and Kawakami, T. (1996). Tec family protein–tyrosine kinases and pleckstrin homology domains in mast cells. *Immunol. Lett.* **54**, 113–117.
- Kawakami, Y., Kitaura, J., Hartman, S. E., Lowell, C. A., Siraganian, R. P., and Kawakami, T. (2000). Regulation of protein kinase C $\beta$ 1 by two protein–tyrosine kinases, Btk and Syk. *Proc. Natl Acad. Sci. USA* **97**, 7423–7428.
- Kim, Y. J., Sekiya, F., Poulin, B., Bae, Y. S., and Rhee, S. G. (2004). Mechanism of B-cell receptor-induced phosphorylation and activation of phospholipase C- $\gamma$ 2. *Mol. Cell. Biol.* **24**, 9986–9999.
- King, N., and Carroll, S. B. (2001). A receptor tyrosine kinase from choanoflagellates: Molecular insights into early animal evolution. *Proc. Natl Acad. Sci. USA* **98**, 15032–15037.
- King, N., Westbrook, M. J., Young, S. L., Kuo, A., Abedin, M., Chapman, J., Fairclough, S., Hellsten, U., Isogai, Y., Letunic, I., Marr, M., Pincus, D., *et al.* (2008). The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. *Nature* **451**, 783–788.
- Knapp, S., Mattsson, P. T., Christova, P., Berndt, K. D., Karshikoff, A., Vihinen, M., Smith, C. I. E., and Ladenstein, R. (1998). Thermal unfolding of small proteins with SH3 domain folding pattern. *Proteins* **31**, 309–319.
- Kojima, T., Fukuda, M., Watanabe, Y., Hamazato, F., and Mikoshiba, K. (1997). Characterization of the pleckstrin homology domain of Btk as an inositol polyphosphate and phosphoinositide binding domain. *Biochem. Biophys. Res. Commun.* **236**, 333–339.
- Korpi, M., Väliäho, J., and Vihinen, M. (2000). Structure-function effects in primary immunodeficiencies. *Scand. J. Immunol.* **52**, 226–232.
- Koyanagi, M., Ono, K., Suga, H., Iwabe, N., and Miyata, T. (1998). Phospholipase C cDNAs from sponge and hydra: Antiquity of genes involved in the inositol phospholipid signaling pathway. *FEBS Lett.* **439**, 66–70.
- Lachance, G., Levesseur, S., and Naccache, P. H. (2002). Chemotactic factor-induced recruitment and activation of Tec family kinases in human neutrophils. Implication of phosphatidylinositol 3-kinases. *J. Biol. Chem.* **277**, 21537–21541.
- Laederach, A., Cradic, K. W., Brazin, K. N., Zmoon, J., Fulton, D. B., Huang, X. Y., and Andreotti, A. H. (2002). Competing modes of self-association in the regulatory domains of Bruton's tyrosine kinase: Intramolecular contact versus asymmetric homodimerization. *Protein Sci.* **11**, 36–45.
- Laederach, A., Cradic, K. W., Fulton, D. B., and Andreotti, A. H. (2003). Determinants of intra versus intermolecular self-association within the regulatory domains of Rlk and Itk. *J. Mol. Biol.* **329**, 1011–1020.

- Lappalainen, I., Thusberg, J., Shen, B., and Vihinen, M. (2008). Genome wide analysis of pathogenic SH2 domain mutations. *Proteins* **72**, 779–792.
- Lee, S. H., Kim, T., Jeong, D., Kim, N., and Choi, Y. (2008). The Tec family tyrosine kinase Btk regulates RANKL-induced osteoclast maturation. *J. Biol. Chem.* **283**, 11526–11534.
- Lehnes, K., Winder, A. D., Alfonso, C., Kasid, N., Simoneaux, M., Summe, H., Morgan, E., Iann, M. C., Duncan, J., Eagan, M., Tavaluc, R., Evans, C. H., Jr., et al. (2007). The effect of estradiol on *in vivo* tumorigenesis is modulated by the human epidermal growth factor receptor 2/phosphatidylinositol 3-kinase/Akt1 pathway. *Endocrinology* **148**, 1171–1180.
- Leitges, M., Schmedt, C., Guinamard, R., Davoust, J., Schaal, S., Stabel, S., and Tarakhovsky, A. (1996). Immunodeficiency in protein kinase  $c\beta$ -deficient mice. *Science* **273**, 788–791.
- Levin, D. E., Fields, F. O., Kunisawa, R., Bishop, J. M., and Thorner, J. (1990). A candidate protein kinase C gene, PKC1, is required for the *S. cerevisiae* cell cycle. *Cell* **62**, 213–224.
- Lewis, C. M., Broussard, C., Czar, M. J., and Schwartzberg, P. L. (2001). Tec kinases: Modulators of lymphocyte signaling and development. *Curr. Opin. Immunol.* **13**, 317–325.
- Li, W., Young, S. L., King, N., and Miller, W. T. (2008). Signaling properties of a non-metazoan Src kinase and the evolutionary history of Src negative regulation. *J. Biol. Chem.* **283**, 15491–15501.
- Liao, X. C., and Littman, D. R. (1995). Altered T cell receptor signaling and disrupted T cell development in mice lacking Itk. *Immunity* **3**, 757–769.
- Lindvall, J. M., Blomberg, K. E., Väliaho, J., Vargas, L., Heinonen, J. E., Berglöf, A., Mohamed, A. J., Nore, B. F., Vihinen, M., and Smith, C. I. E. (2005). Bruton's tyrosine kinase: Cell biology, sequence conservation, mutation spectrum, siRNA modifications, and expression profiling. *Immunol. Rev.* **203**, 200–215.
- Lu, K. P., Hanes, S. D., and Hunter, T. (1996). A human peptidyl-prolyl isomerase essential for regulation of mitosis. *Nature* **380**, 544–547.
- Lu, Y., Cuevas, B., Gibson, S., Khan, H., LaPushin, R., Imboden, J., and Mills, G. B. (1998). Phosphatidylinositol 3-kinase is required for CD28 but not CD3 regulation of the TEC family tyrosine kinase EMT/ITK/TSK: Functional and physical interaction of EMT with phosphatidylinositol 3-kinase. *J. Immunol.* **161**, 5404–5412.
- Mallis, R. J., Brazin, K. N., Fulton, D. B., and Andreotti, A. H. (2002). Structural characterization of a proline-driven conformational switch within the Itk SH2 domain. *Nat. Struct. Biol.* **9**, 900–905.
- Maniar, H. S., Vihinen, M., Webster, A. D., Nilsson, L., and Smith, C. I. E. (1995). Structural basis for X-linked agammaglobulinemia (XLA): Mutations at interacting Btk residues R562, W563, and A582. *Clin. Immunol. Immunopathol.* **76**, S198–S202.
- Manna, D., Albanese, A., Park, W. S., and Cho, W. (2007). Mechanistic basis of differential cellular responses of phosphatidylinositol 3,4-bisphosphate- and phosphatidylinositol 3,4,5-trisphosphate-binding pleckstrin homology domains. *J. Biol. Chem.* **282**, 32093–32105.
- Mano, H., Ishikawa, F., Nishida, J., Hirai, H., and Takaku, F. (1990). A novel protein-tyrosine kinase, tec, is preferentially expressed in liver. *Oncogene* **5**, 1781–1786.
- Mano, H., Mano, K., Tang, B., Koehler, M., Yi, T., Gilbert, D. J., Jenkins, N. A., Copeland, N. G., and Ihle, J. N. (1993). Expression of a novel form of Tec kinase in hematopoietic cells and mapping of the gene to chromosome 5 near Kit. *Oncogene* **8**, 417–424.
- Mansell, A., Smith, R., Doyle, S. L., Gray, P., Fenner, J. E., Crack, P. J., Nicholson, S. E., Hilton, D. J., O'Neill, L. A., and Hertzog, P. J. (2006). Suppressor of cytokine signaling 1 negatively regulates Toll-like receptor signaling by mediating Mal degradation. *Nat. Immunol.* **7**, 148–155.
- Mao, C., Zhou, M., and Uckun, F. M. (2001). Crystal structure of Bruton's tyrosine kinase domain suggests a novel pathway for activation and provides insights into the molecular basis of X-linked agammaglobulinemia. *J. Biol. Chem.* **276**, 41435–41443.

- Márquez, J. A., Smith, C. I. E., Petoukhov, M. V., Lo Surdo, P., Mattsson, P. T., Knekt, M., Westlund, A., Scheffzek, K., Saraste, M., and Svergun, D. I. (2003). Conformation of full-length Bruton tyrosine kinase (Btk) from synchrotron X-ray solution scattering. *EMBO J.* **22**, 4616–4624.
- Matsushita, M., Yamadori, T., Kato, S., Takemoto, Y., Inazawa, J., Baba, Y., Hashimoto, S., Sekine, S., Arai, S., Kunikata, T., Kurimoto, M., Kishimoto, T., *et al.* (1998). Identification and characterization of a novel SH3-domain binding protein, Sab, which preferentially associates with Bruton's tyrosine kinase (BTK). *Biochem. Biophys. Res. Commun.* **245**, 337–343.
- Mattsson, P. T., Vihinen, M., and Smith, C. I. E. (1996). X-linked agammaglobulinemia (XLA): A genetic tyrosine kinase (Btk) disease. *BioEssays* **18**, 825–834.
- Mattsson, P. T., Lappalainen, I., Bäckesjö, C. M., Brockmann, E., Lauren, S., Vihinen, M., and Smith, C. I. E. (2000). Six X-linked agammaglobulinemia-causing missense mutations in the Src homology 2 domain of Bruton's tyrosine kinase: Phosphotyrosine-binding and circular dichroism analysis. *J. Immunol.* **164**, 4170–4177.
- Melcher, M., Unger, B., Schmidt, U., Rajantie, I. A., Alitalo, K., and Ellmeier, W. (2008). Essential roles for the Tec family kinases Tec and Btk in M-CSF receptor signaling pathways that regulate macrophage survival. *J. Immunol.* **180**, 8048–8056.
- Moarefi, I., LaFevre-Bernt, M., Sicheri, F., Huse, M., Lee, C. H., Kuriyan, J., and Miller, W. T. (1997). Activation of the Src-family tyrosine kinase Hck by SH3 domain displacement. *Nature* **385**, 650–653.
- Nars, M., and Vihinen, M. (2001). Coevolution of the domains of cytoplasmic tyrosine kinases. *Mol. Biol. Evol.* **18**, 312–321.
- Nore, B. F., Vargas, L., Mohamed, A. J., Brandén, L. J., Bäckesjö, C. M., Islam, T. C., Mattsson, P. T., Hultenby, K., Christensson, B., and Smith, C. I. E. (2000). Redistribution of Bruton's tyrosine kinase by activation of phosphatidylinositol 3-kinase and Rho-family GTPases. *Eur. J. Immunol.* **30**, 145–154.
- Nore, B. F., Mattsson, P. T., Antonsson, P., Bäckesjö, C. M., Westlund, A., Lennartsson, J., Hansson, H., Low, P., Rönstrand, L., and Smith, C. I. E. (2003). Identification of phosphorylation sites within the SH3 domains of Tec family tyrosine kinases. *Biochim. Biophys. Acta* **1645**, 123–132.
- Notarangelo, L. D., Mella, P., Jones, A., de Saint Basile, G., Savoldi, G., Cranston, T., Vihinen, M., and Schumacher, R. F. (2001). Mutations in severe combined immune deficiency (SCID) due to JAK3 deficiency. *Hum. Mutat.* **18**, 255–263.
- Oda, A., Ikeda, Y., Ochs, H. D., Druker, B. J., Ozaki, K., Handa, M., Ariga, T., Sakiyama, Y., Witte, O. N., and Wahl, M. I. (2000). Rapid tyrosine phosphorylation and activation of Bruton's tyrosine/Tec kinases in platelets induced by collagen binding or CD32 cross-linking. *Blood* **95**, 1663–1670.
- Okoh, M. P., and Vihinen, M. (1999). Pleckstrin homology domains of tec family protein kinases. *Biochem. Biophys. Res. Commun.* **265**, 151–157.
- Okoh, M. P., Kainulainen, L., Heiskanen, K., Isa, M. N., Varming, K., Ruuskanen, O., and Vihinen, M. (2002). Novel insertions of Bruton tyrosine kinase in patients with X-linked agammaglobulinemia. *Hum. Mutat.* **20**, 480–481.
- Okoh, M. P., and Vihinen, M. (2002). Interaction between Btk TH and SH3 domain. *Biopolymers* **63**, 325–334.
- Ortutay, C., Väliäho, J., Stenberg, K., and Vihinen, M. (2005). KinMutBase: A registry of disease-causing mutations in protein kinase domains. *Hum. Mutat.* **25**, 435–442.
- Park, H., Wahl, M. I., Afar, D. E., Turck, C. W., Rawlings, D. J., Tam, C., Scharenberg, A. M., Kinet, J. P., and Witte, O. N. (1996). Regulation of Btk function by a major autophosphorylation site within the SH3 domain. *Immunity* **4**, 515–525.



- Park, W. S., Heo, W. D., Whalen, J. H., O'Rourke, N. A., Bryan, H. M., Meyer, T., and Teruel, M. N. (2008). Comprehensive identification of PIP3-regulated PH domains from *C. elegans* to *H. sapiens* by model prediction and live imaging. *Mol. Cell* **30**, 381–392.
- Pawson, T., and Scott, J. D. (2005). Protein phosphorylation in signaling-50 years and counting. *Trends Biochem. Sci.* **30**, 286–290.
- Pawson, T., Gish, G. D., and Nash, P. (2001). SH2 domains, interaction modules and cellular wiring. *Trends Cell Biol.* **11**, 504–511.
- Peterson, K. J., and Butterfield, N. J. (2005). Origin of the Eumetazoa: Testing ecological predictions of molecular clocks against the Proterozoic fossil record. *Proc. Natl Acad. Sci. USA* **102**, 9547–9552.
- Petro, J. B., Rahman, S. M., Ballard, D. W., and Khan, W. N. (2000). Bruton's tyrosine kinase is required for activation of I $\kappa$ B kinase and nuclear factor  $\kappa$ B in response to B cell receptor engagement. *J. Exp. Med.* **191**, 1745–1754.
- Piirilä, H., Väliäho, J., and Vihinen, M. (2006). Immunodeficiency mutation databases (IDbases). *Hum. Mutat.* **27**, 1200–1208.
- Pincus, D., Letunic, I., Bork, P., and Lim, W. A. (2008). Evolution of the phospho-tyrosine signaling machinery in premetazoan lineages. *Proc. Natl. Acad. Sci. USA* **105**, 9680–9684.
- Pursglove, S. E., Mulhern, T. D., Mackay, J. P., Hinds, M. G., and Booker, G. W. (2002). The solution structure and intramolecular associations of the Tec kinase Src homology 3 domain. *J. Biol. Chem.* **277**, 755–762.
- Putnam, N. H., Butts, T., Ferrier, D. E., Furlong, R. F., Hellsten, U., Kawashima, T., Robinson-Rechavi, M., Shoguchi, E., Terry, A., Yu, J. K., Benito-Gutierrez, E. L., Dubchak, I., et al. (2008). The amphioxus genome and the evolution of the chordate karyotype. *Nature* **453**, 1064–1071.
- Qiu, Y., Robinson, D., Pretlow, T. G., and Kung, H. J. (1998). Etk/Bmx, a tyrosine kinase with a pleckstrin-homology domain, is an effector of phosphatidylinositol 3'-kinase and is involved in interleukin 6-induced neuroendocrine differentiation of prostate cancer cells. *Proc. Natl Acad. Sci. USA* **95**, 3644–3649.
- Quintaje, S. B., and Orchard, S. (2008). The annotation of both human and mouse kinomes in UniProtKB/Swiss-Prot: One small step in manual annotation, one giant step for full comprehension of genomes. *Mol. Cell Proteomics* **7**(8), 1409–1419.
- Rajantie, I., Ekman, N., Iljin, K., Arighi, E., Gunji, Y., Kaukonen, J., Palotie, A., Dewerchin, M., Carmeliet, P., and Alitalo, K. (2001). Bmx tyrosine kinase has a redundant function downstream of angiopoietin and vascular endothelial growth factor receptors in arterial endothelium. *Mol. Cell Biol.* **21**, 4647–4655.
- Rameh, L. E., Arvidsson, A., Carraway, K. L., III, Couvillon, A. D., Rathbun, G., Crompton, A., VanRenterghem, B., Czech, M. P., Ravichandran, K. S., Burakoff, S. J., Wang, D. S., Chen, C. S., et al. (1997). A comparative analysis of the phosphoinositide binding specificity of pleckstrin homology domains. *J. Biol. Chem.* **272**, 22059–22066.
- Rawlings, D. J., Saffran, D. C., Tsukada, S., Largaespada, D. A., Grimaldi, J. C., Cohen, L., Mohr, R. N., Bazan, J. F., Howard, M., Copeland, N. G., et al. (1993). Mutation of unique region of Bruton's tyrosine kinase in immunodeficient XID mice. *Science* **261**, 358–361.
- Robinson, D., He, F., Pretlow, T., and Kung, H. J. (1996). A tyrosine kinase profile of prostate carcinoma. *Proc. Natl Acad. Sci. USA* **93**, 5958–5962.
- Roulier, E. M., Panzer, S., and Beckendorf, S. K. (1998). The Tec29 tyrosine kinase is required during *Drosophila* embryogenesis and interacts with Src64 in ring canal development. *Mol. Cell* **1**, 819–829.
- Sable, C. L., Filippa, N., Filloux, C., Hemmings, B. A., and Van Obberghen, E. (1998). Involvement of the pleckstrin homology domain in the insulin-stimulated activation of protein kinase B. *J. Biol. Chem.* **273**, 29600–29606.

- Saharinen, P., Ekman, N., Sarvas, K., Parker, P., Alitalo, K., and Silvennoinen, O. (1997). The Bmx tyrosine kinase induces activation of the Stat signaling pathway, which is specifically inhibited by protein kinase C $\delta$ . *Blood* **90**, 4341–4353.
- Saito, K., Scharenberg, A. M., and Kinet, J. P. (2001). Interaction between the Btk PH domain and phosphatidylinositol-3,4,5-trisphosphate directly regulates Btk. *J. Biol. Chem.* **276**, 16201–16206.
- Salim, K., Bottomley, M. J., Querfurth, E., Zvelebil, M. J., Gout, I., Scaife, R., Margolis, R. L., Gigg, R., Smith, C. I. E., Driscoll, P. C., Waterfield, M. D., and Panayotou, G. (1996). Distinct specificity in the recognition of phosphoinositides by the pleckstrin homology domains of dynamin and Bruton's tyrosine kinase. *EMBO J.* **15**, 6241–6250.
- Schaeffer, E. M., Debnath, J., Yap, G., McVicar, D., Liao, X. C., Littman, D. R., Sher, A., Varmus, H. E., Lenardo, M. J., and Schwartzberg, P. L. (1999). Requirement for Tec kinases Rlk and Itk in T cell receptor signaling and immunity. *Science* **284**, 638–641.
- Schieven, G., Thorner, J., and Martin, G. S. (1986). Protein–tyrosine kinase activity in *Saccharomyces cerevisiae*. *Science* **231**, 390–393.
- Schmidt, U., van den Akker, E., Paren-van Amelsvoort, M., Litos, G., de Bruijn, M., Gutierrez, L., Hendriks, R. W., Ellmeier, W., Lowenberg, B., Beug, H., and von Lindern, M. (2004a). Btk is required for an efficient response to erythropoietin and for SCF-controlled protection against TRAIL in erythroid progenitors. *J. Exp. Med.* **199**, 785–795.
- Schmidt, U., Boucheron, N., Unger, B., and Ellmeier, W. (2004b). The role of Tec family kinases in myeloid cells. *Int. Arch. Allergy Immunol.* **134**, 65–78.
- Seet, B. T., Dikic, I., Zhou, M. M., and Pawson, T. (2006). Reading protein modifications with interaction domains. *Nat. Rev. Mol. Cell Biol.* **7**, 473–483.
- Segawa, Y., Suga, H., Iwabe, N., Oneyama, C., Akagi, T., Miyata, T., and Okada, M. (2006). Functional development of Src tyrosine kinases during evolution from a unicellular ancestor to multicellular animals. *Proc. Natl Acad. Sci. USA* **103**, 12021–12026.
- Serfas, M. S., and Tyner, A. L. (2003). Brk, Srm, Frk, and Src42A form a distinct family of intracellular Src-like tyrosine kinases. *Oncol. Res.* **13**, 409–419.
- Severin, A., Fulton, D. B., and Andreotti, A. H. (2008). Murine Itk SH3 domain. *J. Biomol. NMR* **40**, 285–290.
- Shinohara, M., Koga, T., Okamoto, K., Sakaguchi, S., Arai, K., Yasuda, H., Takai, T., Kodama, T., Morio, T., Geha, R. S., Kitamura, D., Kurosaki, T., *et al.* (2008). Tyrosine kinases Btk and Tec regulate osteoclast differentiation by linking RANK and ITAM signals. *Cell* **132**, 794–806.
- Sicheri, F., Moarefi, I., and Kuriyan, J. (1997). Crystal structure of the Src family tyrosine kinase Hck. *Nature* **385**, 602–609.
- Siliciano, J. D., Morrow, T. A., and Desiderio, S. V. (1992). ITK, a T-cell-specific tyrosine kinase gene inducible by interleukin 2. *Proc. Natl Acad. Sci. USA* **89**, 11194–11198.
- Sinka, R., Jankovics, F., Somogyi, K., Szlanka, T., Lukacsovich, T., and Erdelyi, M. (2002). *poirot*, a new regulatory gene of *Drosophila* oskar acts at the level of the short Oskar protein isoform. *Development* **129**, 3469–3478.
- Smith, C. I. E., Baskin, B., Humire-Greiff, P., Zhou, J. N., Olsson, P. G., Maniar, H. S., Kjellen, P., Lambris, J. D., Christensson, B., Hammarström, L., *et al.* (1994a). Expression of Bruton's agammaglobulinemia tyrosine kinase gene, BTK, is selectively down-regulated in T lymphocytes and plasma cells. *J. Immunol.* **152**, 557–565.
- Smith, C. I. E., Islam, K. B., Vorechovsky, I., Olerup, O., Wallin, E., Rabbani, H., Baskin, B., and Hammarström, L. (1994b). X-linked agammaglobulinemia and other immunoglobulin deficiencies. *Immunol. Rev.* **138**, 159–183.
- Smith, C. I. E., Islam, T. C., Mattsson, P. T., Mohamed, A. J., Nore, B. F., and Vihinen, M. (2001). The Tec family of cytoplasmic tyrosine kinases: Mammalian Btk, Bmx, Itk, Tec, Txk and homologs in other species. *BioEssays* **23**, 436–446.

- Speletas, M., Kanariou, M., Kanakoudi-Tsakalidou, F., Papadopoulou-Alataki, E., Arvanitidis, K., Pardali, E., Constantopoulos, A., Kartalis, G., Vihinen, M., Sideras, P., and Ritis, K. (2001). Analysis of Btk mutations in patients with X-linked agammaglobulinaemia (XLA) and determination of carrier status in normal female relatives: A nationwide study of Btk deficiency in Greece. *Scand. J. Immunol.* **54**, 321–327.
- Sprague, J., Bayraktaroglu, L., Bradford, Y., Conlin, T., Dunn, N., Fashena, D., Frazer, K., Haendel, M., Howe, D. G., Knight, J., Mani, P., Moxon, S. A., *et al.* (2008). The Zebrafish Information Network: The zebrafish model organism database provides expanded support for genotypes and phenotypes. *Nucleic Acids Res.* **36**, D768–D772.
- Stephens, L. R., Jackson, T. R., and Hawkins, P. T. (1993). Agonist-stimulated synthesis of phosphatidylinositol(3,4,5)-trisphosphate: A new intracellular signalling system? *Biochim. Biophys. Acta* **1179**, 27–75.
- Stoica, G. E., Franke, T. F., Moroni, M., Mueller, S., Morgan, E., Iann, M. C., Winder, A. D., Reiter, R., Wellstein, A., Martin, M. B., and Stoica, A. (2003). Effect of estradiol on estrogen receptor-alpha gene expression and activity can be modulated by the ErbB2/PI 3-K/Akt pathway. *Oncogene* **22**, 7998–8011.
- Strausberg, R. L., Feingold, E. A., Grouse, L. H., Derge, J. G., Klausner, R. D., Collins, F. S., Wagner, L., Shenmen, C. M., Schuler, G. D., Altschul, S. F., Zeeberg, B., Buetow, K. H., *et al.* (2002). Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proc. Natl. Acad. Sci. USA* **99**, 16899–16903.
- Swofford, D. (2003). “PAUP\*. Phylogenetic Analysis Using Parsimony (\* and Other Methods). Version 4.” Sinauer Associates, Sunderland, MA.
- Tamagnone, L., Lahtinen, I., Mustonen, T., Virtaneva, K., Francis, F., Muscatelli, F., Alitalo, R., Smith, C. I. E., Larsson, C., and Alitalo, K. (1994). BMX, a novel nonreceptor tyrosine kinase gene of the BTK/ITK/TEC/TXK family located in chromosome Xp22.2. *Oncogene* **9**, 3683–3688.
- Tasma, I. M., Brendel, V., Whitham, S. A., and Bhattacharyya, M. K. (2008). Expression and evolution of the phosphoinositide-specific phospholipase C gene family in *Arabidopsis thaliana*. *Plant Physiol. Biochem.* **46**, 627–637.
- Thomas, J. H., and Wieschaus, E. (2004). src64 and tec29 are required for microfilament contraction during *Drosophila* cellularization. *Development* **131**, 863–871.
- Thomas, J. D., Sideras, P., Smith, C. I. E., Vorechovsky, I., Chapman, V., and Paul, W. E. (1993). Colocalization of X-linked agammaglobulinemia and X-linked immunodeficiency genes. *Science* **261**, 355–358.
- Tomlinson, M. G., Heath, V. L., Turck, C. W., Watson, S. P., and Weiss, A. (2004). SHIP family inositol phosphatases interact with and negatively regulate the Tec tyrosine kinase. *J. Biol. Chem.* **279**, 55089–55096.
- Tsakada, S., Saffran, D. C., Rawlings, D. J., Parolini, O., Allen, R. C., Klisak, I., Sparkes, R. S., Kubagawa, H., Mohandas, T., Quan, S., *et al.* (1993). Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* **72**, 279–290.
- Uckun, F. M. (1998). Bruton’s tyrosine kinase (BTK) as a dual-function regulator of apoptosis. *Biochem. Pharmacol.* **56**, 683–691.
- Väliaho, J., Smith, C. I. E., and Vihinen, M. (2006). BTKbase: The mutation database for X-linked agammaglobulinemia. *Hum. Mutat.* **27**, 1209–1217.
- Vihinen, M., and Durandy, A. (2006). Primary Immunodeficiencies: Genotype-Phenotype Correlations. In “Immunogenomics and Human Disease” (A. Falus, ed.). John Wiley & Sons Inc., Hoboken, New Jersey. Vol. 1, pp. 443–460.
- Vihinen, M., Villa, A., Mella, P., Schumacher, R. F., Savoldi, G., O’Shea, J. J., Candotti, F., and Notarangelo, L. D. (2000). Molecular modeling of the Jak3 kinase domains and structural basis for severe combined immunodeficiency. *Clin. Immunol.* **96**, 108–118.

- Vorechovsky, I., Vihinen, M., de Saint Basile, G., Honsova, S., Hammarstrom, L., Muller, S., Nilsson, L., Fischer, A., and Smith, C. I. (1995). DNA-based mutation analysis of Bruton's tyrosine kinase gene in patients with X-linked agammaglobulinaemia. *Hum. Mol. Genet.* **4**, 51–58.
- van Dijk, T. B., van Den Akker, E., Amelvoort, M. P., Mano, H., Lowenberg, B., and von Lindern, M. (2000). Stem cell factor induces phosphatidylinositol 3'-kinase-dependent Lyn/Tec/Dok-1 complex formation in hematopoietic cells. *Blood* **96**, 3406–3413.
- Vanhaesebroeck, B., Leever, S. J., Panayotou, G., and Waterfield, M. D. (1997). Phosphoinositide 3-kinases: A conserved family of signal transducers. *Trends Biochem. Sci.* **22**, 267–272.
- Varnai, P., Bondeva, T., Tamas, P., Toth, B., Buday, L., Hunyady, L., and Balla, T. (2005). Selective cellular effects of overexpressed pleckstrin-homology domains that recognize Ptdins(3,4,5)P<sub>3</sub> suggest their interaction with protein binding partners. *J. Cell Sci.* **118**, 4879–4888.
- Venkataraman, C., Chen, X. C., Na, S., Lee, L., Neote, K., and Tan, S. L. (2006). Selective role of PKC $\beta$  enzymatic function in regulating cell survival mediated by B cell antigen receptor cross-linking. *Immunol. Lett.* **105**, 83–89.
- Vetrie, D., Vorechovsky, I., Sideras, P., Holland, J., Davies, A., Flinter, F., Hammarström, L., Kinnon, C., Levinsky, R., Bobrow, M., *et al.* (1993). The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. *Nature* **361**, 226–233.
- Vihinen, M., Nilsson, L., and Smith, C. I. E. (1994a). Tec homology (TH) adjacent to the PH domain. *FEBS Lett.* **350**, 263–265.
- Vihinen, M., Nilsson, L., and Smith, C. I. E. (1994b). Structural basis of SH2 domain mutations in X-linked agammaglobulinemia. *Biochem. Biophys. Res. Commun.* **205**, 1270–1277.
- Vihinen, M., Vetrie, D., Maniar, H. S., Ochs, H. D., Zhu, Q., Vorechovsky, I., Webster, A. D., Notarangelo, L. D., Nilsson, L., Sowadski, J. M., *et al.* (1994c). Structural basis for chromosome X-linked agammaglobulinemia: A tyrosine kinase disease. *Proc. Natl Acad. Sci. USA* **91**, 12803–12807.
- Vihinen, M., Cooper, M. D., de Saint Basile, G., Fischer, A., Good, R. A., Hendriks, R. W., Kinnon, C., Kwan, S. P., Litman, G. W., Notarangelo, L. D., *et al.* (1995a). BTKbase: A database of XLA-causing mutations. *Immunol. Today* **16**, 460–465.
- Vihinen, M., Zvelebil, M. J., Zhu, Q., Brooimans, R. A., Ochs, H. D., Zegers, B. J., Nilsson, L., Waterfield, M. D., and Smith, C. I. E. (1995b). Structural basis for pleckstrin homology domain mutations in X-linked agammaglobulinemia. *Biochemistry* **34**, 1475–1481.
- Vihinen, M., Iwata, T., Kinnon, C., Kwan, S. P., Ochs, H. D., Vorechovsky, I., and Smith, C. I. E. (1996). BTKbase, mutation database for X-linked agammaglobulinemia (XLA). *Nucleic Acids Res.* **24**, 160–165.
- Vihinen, M., Nore, B. F., Mattsson, P. T., Bäckesjö, C. M., Nars, M., Koutaniemi, S., Watanabe, C., Lester, T., Jones, A., Ochs, H. D., and Smith, C. I. E. (1997a). Missense mutations affecting a conserved cysteine pair in the TH domain of Btk. *FEBS Lett.* **413**, 205–210.
- Vihinen, M., Belohradsky, B. H., Haire, R. N., Holinski-Feder, E., Kwan, S. P., Lappalainen, I., Lehväsliho, H., Lester, T., Meindl, A., Ochs, H. D., Ollila, J., Vorechovsky, I., *et al.* (1997b). BTKbase, mutation database for X-linked agammaglobulinemia (XLA). *Nucleic Acids Res.* **25**, 166–171.
- Vihinen, M., Brandau, O., Brandén, L. J., Kwan, S. P., Lappalainen, I., Lester, T., Noordzij, J. G., Ochs, H. D., Ollila, J., Pienaar, S. M., Riikonen, P., Saha, B. K., *et al.* (1998). BTKbase, mutation database for X-linked agammaglobulinemia (XLA). *Nucleic Acids Res.* **26**, 242–247.
- Vihinen, M., Kwan, S. P., Lester, T., Ochs, H. D., Resnick, I., Väliaho, J., Conley, M. E., and Smith, C. I. E. (1999). Mutations of the human BTK gene coding for Bruton tyrosine kinase in X-linked agammaglobulinemia. *Hum. Mutat.* **13**, 280–285.

- Vihinen, M., Arredondo-Vega, F. X., Casanova, J. L., Etzioni, A., Giliani, S., Hammarström, L., Hershfield, M. S., Heyworth, P. G., Hsu, A. P., Lähdesmäki, A., Lappalainen, I., Notarangelo, L. D., *et al.* (2001). Primary immunodeficiency mutation databases. *Adv. Genet.* **43**, 103–188.
- Vorechovsky, I., Luo, L., Hertz, J. M., Frøland, S. S., Klemola, T., Fiorini, M., Quinti, I., Paganelli, R., Ozsahin, H., Hammarström, L., Webster, A. D., and Smith, C. I. E. (1997). Mutation pattern in the Bruton's tyrosine kinase gene in 26 unrelated patients with X-linked agammaglobulinemia. *Hum. Mutat.* **9**, 418–425.
- Wang, Q., Deloia, M. A., Kang, Y., Litchke, C., Zhang, N., Titus, M. A., and Walters, K. J. (2007). The SH3 domain of a M7 interacts with its C-terminal proline-rich region. *Protein Sci.* **16**, 189–196.
- Watanabe, N., Nakajima, H., Suzuki, H., Oda, A., Matsubara, Y., Moroi, M., Terauchi, Y., Kadowaki, T., Koyasu, S., Ikeda, Y., and Handa, M. (2003). Functional phenotype of phosphoinositide 3-kinase p85 $\alpha$ -null platelets characterized by an impaired response to GP VI stimulation. *Blood* **102**, 541–548.
- Welters, P., Takegawa, K., Emr, S. D., and Chrispeels, M. J. (1994). AtVSP34, a phosphatidylinositol 3-kinase of *Arabidopsis thaliana*, is an essential protein with homology to a calcium-dependent lipid binding domain. *Proc. Natl. Acad. Sci. USA* **91**, 11398–11402.
- Wilcox, H. M., and Berg, L. J. (2003). Itk phosphorylation sites are required for functional activity in primary T cells. *J. Biol. Chem.* **278**, 37112–37121.
- Williams, J. G., and Zvelebil, M. (2004). SH2 domains in plants imply new signalling scenarios. *Trends Plant Sci.* **9**, 161–163.
- Williams, J. C., Weijland, A., Gonfloni, S., Thompson, A., Courtneidge, S. A., Superti-Furga, G., and Wierenga, R. K. (1997). The 2.35 Å crystal structure of the inactivated form of chicken Src: A dynamic molecule with multiple regulatory interactions. *J. Mol. Biol.* **274**, 757–775.
- Wiltshire, C., Matsushita, M., Tsukada, S., Gillespie, D. A., and May, G. H. (2002). A new c-Jun N-terminal kinase (JNK)-interacting protein, Sab (SH3BP5), associates with mitochondria. *Biochem. J.* **367**, 577–585.
- Wiltshire, C., Gillespie, D. A., and May, G. H. (2004). Sab (SH3BP5), a novel mitochondria-localized JNK-interacting protein. *Biochem. Soc. Trans.* **32**, 1075–1077.
- Xu, W., Harrison, S. C., and Eck, M. J. (1997). Three-dimensional structure of the tyrosine kinase c-Src. *Nature* **385**, 595–602.
- Xue, L. Y., Qiu, Y., He, J., Kung, H. J., and Oleinick, N. L. (1999). Etk/Bmx, a PH-domain containing tyrosine kinase, protects prostate cancer cells from apoptosis induced by photodynamic therapy or thapsigargin. *Oncogene* **18**, 3391–3398.
- Yamada, N., Kawakami, Y., Kimura, H., Fukamachi, H., Baier, G., Altman, A., Kato, T., Inagaki, Y., and Kawakami, T. (1993). Structure and expression of novel protein-tyrosine kinases, Emb and Emt, in hematopoietic cells. *Biochem. Biophys. Res. Commun.* **192**, 231–240.
- Yamadori, T., Baba, Y., Matsushita, M., Hashimoto, S., Kurosaki, M., Kurosaki, T., Kishimoto, T., and Tsukada, S. (1999). Bruton's tyrosine kinase activity is negatively regulated by Sab, the Btk-SH3 domain-binding protein. *Proc. Natl. Acad. Sci. USA* **96**, 6341–6346.
- Yang, X. L., Zhang, Y. L., Lai, Z. S., Xing, F. Y., and Liu, Y. H. (2003). Pleckstrin homology domain of G protein-coupled receptor kinase-2 binds to PKC and affects the activity of PKC kinase. *World J. Gastroenterol.* **9**, 800–803.
- Yao, L., Kawakami, Y., and Kawakami, T. (1994). The pleckstrin homology domain of Bruton tyrosine kinase interacts with protein kinase C. *Proc. Natl. Acad. Sci. USA* **91**, 9175–9179.
- Yao, J. L., Kops, O., Lu, P. J., and Lu, K. P. (2001). Functional conservation of phosphorylation-specific prolyl isomerases in plants. *J. Biol. Chem.* **276**, 13517–13523.

- Yu, L., Mohamed, A. J., Vargas, L., Berglöf, A., Finn, G., Lu, K. P., and Smith, C. I. E. (2006). Regulation of Bruton tyrosine kinase by the peptidylprolyl isomerase Pin1. *J. Biol. Chem.* **281**, 18201–18207.
- Yu, L., Mohamed, A. J., Simonson, O. E., Vargas, L., Blomberg, K. E., Bjorkstrand, B., Arteaga, H. J., Nore, B. F., and Smith, C. I. E. (2008). Proteasome-dependent autoregulation of Bruton tyrosine kinase (Btk) promoter via NF- $\kappa$ B. *Blood* **111**, 4617–4626.
- Zegzouti, H., Li, W., Lorenz, T. C., Xie, M., Payne, C. T., Smith, K., Glenny, S., Payne, G. S., and Christensen, S. K. (2006). Structural and functional insights into the regulation of *Arabidopsis* AGC VIIIa kinases. *J. Biol. Chem.* **281**, 35520–35530.
- Zhu, Q., Zhang, M., Rawlings, D. J., Vihinen, M., Hagemann, T., Saffran, D. C., Kwan, S. P., Nilsson, L., Smith, C. I. E., Witte, O. N., Chen, S. H., and Ochs, H. D. (1994). Deletion within the Src homology domain 3 of Bruton's tyrosine kinase resulting in X-linked agammaglobulinemia (XLA). *J. Exp. Med.* **180**, 461–470.