Beyond calcium: new signaling pathways for Tec family kinases

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Journal of Cell Science 115, 3039-3048 (2002)

Summary

The Tec kinases represent the second largest family of mammalian non-receptor tyrosine kinases and are distinguished by the presence of distinct proline-rich regions and pleckstrin homology domains that are required for proper regulation and activation. Best studied in lymphocyte and mast cells, these kinases are critical for the full activation of phospholipase-C γ (PLC-γ) and Ca^{2+} mobilization downstream of antigen receptors. However, it has become increasingly clear that these kinases are activated downstream of many cell-surface receptors, including receptor tyrosine kinases, cytokine receptors, integrins and G-protein-coupled receptors. Evidence suggests that the Tec kinases influence a wide range of signaling pathways controlling activation of MAP kinases, actin reorganization, transcriptional regulation, cell survival and cellular transformation. Their impact on cellular physiology suggests that the Tec kinases help regulate multiple cellular processes beyond Ca^{2+} mobilization.

Key words: Tyrosine kinase, Pleckstrin homology domain, Phospholipase C-γ, Calcium mobilization, Actin cytoskeleton

Introduction

The Tec kinases first came to the forefront in 1993 when several groups reported that mutations affecting a new tyrosine kinase, Btk, were associated with X-linked agammaglobulinemia (Tsukada et al., 1993; Vetrie et al., 1993), a rare human genetic disorder characterized by severe reductions in serum immunoglobulin levels associated with defective B cell development and function (Vihinen et al., 2000). A similar but less severe phenotype in the mouse *xid* mutant was also found to be caused by a mutation in *btk* (Thomas et al., 1993). It was then recognized that Btk was a member of a new family of non-receptor tyrosine kinases that is now known to include Tec, Btk, Itk/Emt/Tsk, Bmx/Etk, Dsrc29 and Rlk/Txk (Fig. 1a) (Desiderio, 1993; Smith et al., 2001). Although similar in protein organization to the Src family kinases, the Tec kinases possess two distinctive features: (1) a proline-rich region just upstream of the SH3 domain, which has been found to be involved in intra- and intermolecular regulatory interactions; and (2) a pleckstrin homology (PH) domain, which binds both to other proteins and to phospholipids and is required for molecular activation. To date, the Tec kinases are the only tyrosine kinases that are known to possess PH domains.

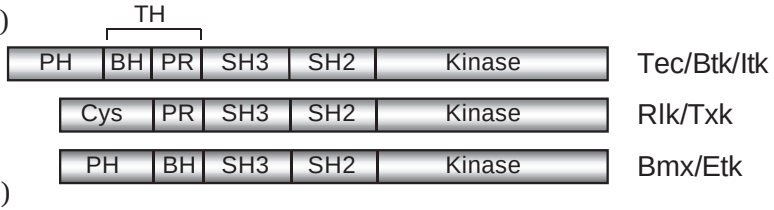
Cells lacking Btk, including cells from XLA patients and mutants of the chicken DT-40 B cell lymphoma line, exhibit defective signaling from the B cell receptor with abnormal activation of PLC-γ and decreased Ca^{2+} mobilization (Fluckiger et al., 1998; Takata and Kurosaki, 1996). Similar results are found in mast and T cells lacking Tec kinases (Kawakami et al., 1999; Liu et al., 1998; Schaeffer et al., 1999). In the past few years, a large body of work has helped examine how Tec kinases participate in the activation of PLC-γ in antigen receptor signaling (Lewis et al., 2001). However, Tec family kinases have also been implicated in multiple signaling pathways from a wide range of cell surface receptors (Qiu and Kung, 2000). Here, we will review how the Tec kinases are activated, their roles in downstream signaling pathways and the consequences of their mutations for cellular physiology with an emphasis on T lymphocyte antigen receptor signaling.

Structure and expression of Tec kinases

Expression of most Tec kinases is restricted to hematopoietic cells with the exception of Etk/Bmx and Tec, which are also expressed in endothelial cells and the liver, respectively (Smith et al., 2001). Btk is found in all cells of hematopoietic lineage except plasma and T cells. By contrast, T cells predominantly express Itk/Emt/Tsk and Rlk/Txk, in addition to Tec. Genetic studies using mice containing mutations of Tec kinases have demonstrated that Tec kinases are required for proper lymphocyte function and development (Lewis et al., 2001; Satterthwaite and Witte, 2000). Lymphocytes can express more than one Tec kinase, and combined mutation of Tec kinases, such as in $Btk^{-/-}Tec^{-/-}$ and $Itk^{-/-}Rlk^{-/-}$ mice, causes more dramatic phenotypes than do single knockouts of each gene (Ellmeier et al., 2000; Schaeffer et al., 1999). These data suggest compensatory roles for Tec kinases in lymphocyte function and development.

Tec kinases possess modular organizations similar to Src family kinases (SFKs), having unique N-termini followed by Src homology 3 (SH3) and Src homology 2 (SH2) protein interaction domains and a tyrosine kinase catalytic domain (SH1) (Fig. 1) (Smith et al., 2001). However, unlike Src kinases, Tec kinases lack N-terminal myristoylation sites and regulatory C-terminal tyrosine residues. Instead, Tec kinases

a)



b)

PH	TH	SH3	SH2	Kinase
InsPtd(3,4,5)P_3	Gα12 (Btk,	Vav	SLP-76	Fas (Btk)
PLC-γ	Tec, Etk)	Sab (Btk)	BLNK/SLP-65	TFII-I (Btk)
PI3K	Gαq (Btk)	Sam68 (Btk)	Vav	WASP (Btk)
Gβγ (Btk)	Gβγ (Btk)	EWS (Btk)	Dok-1 (Tec)	BRDG1 (Tec)
Fas (Btk)	Vav (Tec)	Syk (Btk, Itk)	Cyclophilin A	Grb10/GrbIR (Tec)
PKCβI (Btk)	Kit (Tec)	WASP (Btk, Itk)	(Itk)	PI3K (Tec)
TFII-I (Btk)	LAT (Itk)	Cbl (Btk, Itk)		SLP-76 (Rlk/Txk)
F-actin (Btk)	Grb-2 (Itk)	CD28 (Itk, Tec)		Gβγ (Btk)
FAK (Etk)	Dok-1 (Tec)			Vav (Btk)
STAT3 (Etk)	Lyn, Fyn, Hck			Caveolin (Btk, Etk)
PTPD1 (Etk)	(Btk, Itk, Rlk,			Sak (Tec)
Vav (Btk)	Tec)			
Pak1 (Etk)				
IBtk (Btk)				

Fig. 1. The structure and interacting partners of Tec kinases. (a) The domain structure of the Tec family kinases. The PH domain is involved in protein-protein and protein-phospholipid interactions. The TH domain consists of a BH domain and a PR motif. The SH3 domain binds to proline-rich regions, and in collaboration with the SH2 domain it can engage the PR motif in an intramolecular interaction. The SH2 domain binds to phosphorylated tyrosine residues. The kinase domain has tyrosine-kinase catalytic activity. The atypical Tec kinase, Rlk/Txk, has a cysteine-string motif (Cys) instead of a PH domain and lacks the BH domain. Bmx/Etk lacks the PR motif of the TH domain and has an SH3-like domain. DSrc29 has two isoforms, one of which lacks the PH and TH domains. (b) Molecules that interact with the domains of Tec kinases. Both in vivo and in vitro detected interactions are included. Some binding partners have only been demonstrated for specific Tec kinases, and these are indicated in parentheses.

are distinguished by an N-terminal PH domain and adjacent Tec homology (TH) region, which includes a Btk homology (BH) region and one or two proline-rich regions (PR) (except Rlk/Txk, Bmx/Etk and Dsrc29; see Fig. 1a). These unique features contribute to the regulation of these kinases through protein-protein and protein-lipid interaction and may help determine their varied functions in different signaling pathways. Numerous signaling molecules can interact with each of these domains (Fig. 1b) (Qiu and Kung, 2000).

PH domain

The PH domain is a conserved region that can bind both to proteins, including heterotrimeric G-protein subunits, isoforms of protein kinase C (PKC), transcription factors, F-actin, Vav, Fas, and focal adhesion kinase (FAK), and to phospholipids (Fig. 1b) (Qiu and Kung, 2000). Biochemical analyses demonstrate that the PH domain of Btk preferentially binds to PtdIns(3,4,5)P_3 and Ins(1,3,4,5)P_4 (Kojima et al., 1997; Rameh et al., 1997; Salim et al., 1996). Interactions between the PH domain and phopholipids are critical for regulating membrane targeting of most Tec kinases in response to extracellular stimuli (Fig. 2 and below). Recently, PtdIns(3,4,5)P_3 binding of the PH domain was found to increase Btk kinase activity in vitro (Saito et al., 2001). Moreover, the combined PH-TH region of Btk also influences protein substrate recognition and binding, suggesting that these

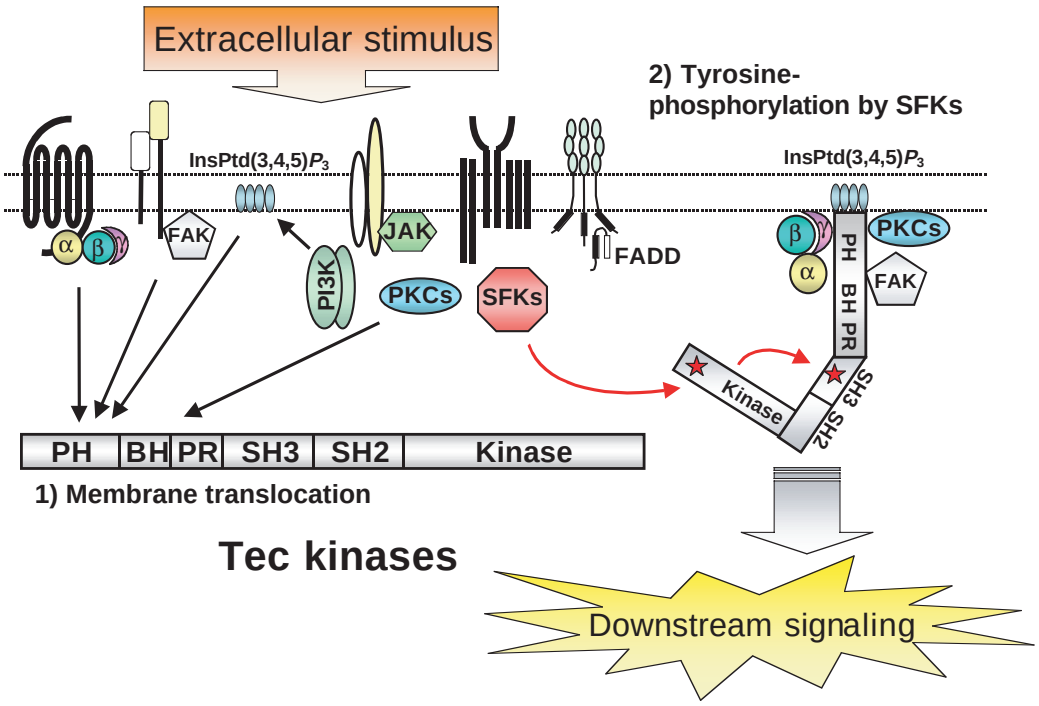


Fig. 2. The two-step activation model for Tec kinases. (1) In the first step, the PH domain interacts with the products of PI3K or, alternatively, with other binding partners (such as the FERM domain of FAK, heterotrimeric G-protein subunits, PKCs or F-actin) to translocate to the plasma membrane or specific intracellular microenvironments required for activation. (2) Once at the membrane, Tec kinases are phosphorylated on a tyrosine residue in their catalytic domain by SFKs. Subsequently, a tyrosine residue in the SH3 domain is autophosphorylated, preventing further inhibitory intramolecular interactions. Phosphorylated tyrosine residues are illustrated as red stars.

domains may play multiple roles in Tec kinase function (Lowry et al., 2001). Two recently identified inhibitors of Btk kinase activity, Ibtk and Bam11, interact with the PH domain of Btk (Kikuchi et al., 2000; Liu et al., 2001).

TH (BH and PR) domain

The Btk homology (BH) domain is characterized by a zinc-binding sequence with homology to Ras-GAP (Smith et al., 2001). Tec kinases also possess one or two proline-rich sequences (PR), which were first shown to be binding partners for the SH3 domains of the Src kinases Fyn, Hck and Lyn (Cheng et al., 1994). However, a second important regulatory function for the PR region has been suggested from NMR studies demonstrating an intramolecular interaction between the PR and SH3 domains of Itk (Andreotti et al., 1997). More recent data suggest that the PR-SH3 interactions can occur in several Tec family kinases, but in some cases there may be a balance between intramolecular and intermolecular interactions (Brazin et al., 2000; Hansson et al., 2001; Laederach et al., 2002; Pursglove et al., 2002). Disruption of these interactions through binding to other molecules may activate Itk and Btk. A conserved serine at the BH-PR boundary is a potential PKC-phosphorylation site, which can alter membrane association and activity of Btk (Kang et al., 2001).

SH3 domain

The SH3 domain was first described as a conserved region of SFK that interacts with proline-rich regions (Pawson and Gish, 1992). In Tec kinases, these domains are important for intramolecular interactions as well as interactions with other signaling molecules. Mutations of the SH3 domain lead to increased transforming activity of an activated allele of Btk, which is consistent with an inhibitory interaction with the PR domain (Afar et al., 1996; Park et al., 1996). For Btk, autophosphorylation of a tyrosine residue (Y233) in the SH3 domain (Park et al., 1996) can change the affinity of the SH3 domain for certain binding partners and may relieve the interaction between the PR and SH3 domains (Morrogh et al., 1999).

SH2 domain

The SH2 domain is a protein interaction domain conserved among many signaling molecules that bind to phosphorylated tyrosine residues in the context of specific peptide sequences (Pawson and Gish, 1992). For the Tec kinases, the SH2 domain is required for interactions with antigen receptor signaling intermediates, SLP76 and BLNK (Bunnell et al., 2000; Hashimoto et al., 1999; Su et al., 1999). Recent evidence suggests that isomerization of a proline in the SH2 domain of Itk by the prolyl-isomerase cyclophilin contributes to regulation of Itk: inhibition of cyclophilin by Cyclosporin A can increase Itk kinase activity (Brazin et al., 2002).

Activation

Among the many receptor-mediated signaling pathways that activate Tec kinases, signaling from antigen receptors on lymphocytes and the FcεRI on mast cells are perhaps best understood. Activation of Tec kinases by these receptors requires two key steps: membrane localization and subsequent tyrosine-phosphorylation by SFKs (Fig. 2) (Lewis et al., 2001). For most Tec kinases, membrane localization is regulated by the interaction of the PH domain with PtdIns(3,4,5)P_3, a product of phosphoinositide 3-kinases (PI3K). A tyrosine residue in the activation loop of the kinase domain of Tec kinases then becomes phosphorylated by SFKs, leading to increased Tec kinase activity.

This activation model has been supported by numerous observations. Mutation of the Src family phosphorylation site or inhibition of Src kinases prevents activation of Tec kinases (Chamorro et al., 2001; Gibson et al., 1996; Heyeck et al., 1997; Rawlings et al., 1996). Mutation of the PH domain of Btk can cause both XLA in human and *xid* in mice and prevents activation of Btk, Itk and Tec in cell lines (August et al., 1997; Li et al., 1997; Satterthwaite and Witte, 2000; Yang et al., 2001), indicating that the PH domain plays a crucial role in regulation of Tec kinase function. Moreover, activation of Btk, Itk and Tec is prevented by inhibitors of PI3K, and Btk kinase activity can be diminished by SH2-containing inositol phosphatase SHIP1, which decreases the levels of PtdIns(3,4,5)P_3 (August et al., 1997; Li et al., 1997; Scharenberg and Kinet, 1998). Likewise, B cells from mice with a targeted mutation of the PI3K p85α subunit have phenotypes similar to those of *xid* and Btk$^{-/-}$ mice (Fruman et al., 1999; Suzuki et al., 1999). Conversely, deficiency of the inositol phosphatase PTEN leads to constitutive membrane association of Itk in the Jurkat T cells (Shan et al., 2000). Note that PI3K p110γ$^{-/-}$ mice have T cell proliferation defects similar to Itk$^{-/-}$ mice, raising the possibility that this G-protein-coupled PI3K may also contribute to activation of Tec kinases in T cells (Sasaki et al., 2000).

Recent evidence suggests that the interaction of the PH domain with PtdIns(3,4,5)P_3 targets Tec kinases to specific membrane microdomains, referred to as Rafts or glycolipid-enriched membranes (GEMs), where signaling molecules convene upon antigen receptor activation (Simons and Ikonen, 1997). Itk translocates to GEMs upon CD3-TCR stimulation in a PtdIns(3,4,5)P_3- and PH-domain-dependent manner (Bunnell et al., 2000; Shan et al., 2000; Woods et al., 2001). Similar results have been found for Btk (Guo et al., 2000). Intriguingly, both Btk and Bmx physically interact with caveolin-1, a primary protein component of caveolae, a glycolipid-enriched membrane compartment (Vargas et al., 2001).

Although most Tec kinases are regulated in this fashion, the atypical Tec kinase Rlk/Txk lacks a PH domain. Rlk is activated by SFKs; however, unlike other Tec kinases, Rlk is activated independently of PI3K (Debnath et al., 1999). Instead, Rlk possesses a repeated cysteine motif (Fig. 1a), which is palmitoylated and can also target Rlk/Txk to GEMs (Czar et al., 2001). Additionally, a shorter form of Rlk, generated by internal translational initiation, lacks this cysteine motif and can localize to the nucleus (Debnath et al., 1999). Upon TCR activation, a fraction of Rlk translocates to the nucleus, which suggests that Rlk may have distinct cytosolic and nuclear functions (Debnath et al., 1999). Although nuclear localization was thought to be a unique feature of Rlk, evidence now suggests that both Btk and Itk can traffic to the nucleus

upon antigen receptor signaling (Perez-Villar et al., 2001; Webb et al., 2000).

Other activation pathways

In addition to antigen receptor signaling, many other receptor signaling pathways induce translocation of Tec kinases to the plasma membrane, leading to kinase activation. Gβγ subunits bind directly to the PHTH domain of Btk in vitro, using purified recombinant proteins, which results in increased kinase activity (Tsukada et al., 1994). More recently, Lowry and co-workers have shown that overexpressed Gβγ induces translocation of Btk to the plasma membrane, and the PH-TH domains of Btk are required for membrane targeting. Interestingly, Gβγ-mediated translocation of Btk is PI3K dependent, which suggests a possible role for a G-protein-regulated PI3K in the translocation of Btk (Lowry et al., 2001; Lowry and Huang, 2002). Similarly, the chemokine SDF-1α and fMet-Leu-Phe, ligands for G-protein-coupled receptors, induce translocation of Btk, Itk and Tec to the plasma membrane in a PI3K-dependent manner (Lachance et al., 2002; Nore et al., 2000) (A.T., unpublished). Indeed, a wide range of receptors that activate PI3Ks, including tyrosine kinase, chemokine and antigen receptors, as well as the direct activation of PI3K, induce membrane translocation of Btk and BTK-PH-GFP fusion proteins (Nore et al., 2000; Varnai et al., 1999).

In addition to Btk and Itk, other Tec kinases also change localization in response to extracellular stimuli. The PH domain of Etk binds to the FERM domain of FAK upon extracellular matrix stimulation of integrins, which leads to the activation of Etk (Chen et al., 2001). Both Etk and Tec can associate with and be activated by a number of receptor tyrosine kinases as well as cytokine receptors (Mano, 1999; Rajantie et al., 2001). Activation of the tyrosine kinase receptor Kit by stem cell factor induces formation of a Lyn-Tec-Dok-1 complex and activation of Tec, both of which are prevented by PI3K inhibition (van Dijk et al., 2000). Stimulation of prolactin receptor (PRLr), a member of the cytokine receptor superfamily, induces activation of Tec and association of both Tec and Vav with the intracellular domain of PRLr (Kline et al., 2001). Together, these observations support a model in which Tec kinases require proper targeting for their activation, probably through the interaction of their PH domains with PtdIns(3,4,5)P_3 or other signaling molecules.

Functions of Tec kinases

Antigen receptor signaling

Antigen receptors on lymphocytes, including surface immunoglobulins on B cells and T cell receptors on T cells, consist of variable rearranged gene products associated with common invariant chains. Although antigen receptors have no intrinsic tyrosine kinase activity, engagement of these receptors leads to the rapid sequential activation of members of the Src and Syk families of tyrosine kinases, which then phosphorylate adaptor molecules, leading to the recruitment of downstream effectors into a signaling complex required for the activation of PLC-γ and MAP kinases, actin reorganization and transcriptional activation (Lin and Weiss, 2001). The Tec kinases appear to be important components of these antigen receptor signaling complexes required for full activation of these downstream pathways (Lewis et al., 2001). In particular, humans, mice and cell lines possessing mutant Btk or Itk and Rlk have defective responses to antigen receptor signaling, exhibiting decreased Ca^{2+} mobilization, activation of MAP kinases, cytoskeleton rearrangements and transcriptional activation, and this leads to altered development and defects in functional responses, including cellular proliferation, expression of activation markers, cytokine and antibody production and responses to infectious disease (Lewis et al., 2001; Satterthwaite and Witte, 2000).

In T cells, initiation of T cell antigen receptor (TCR) signaling rapidly activates the Src kinase Lck, which phosphorylates a series of conserved dual tyrosines, the immunotyrosine activation motifs (ITAMs), on the invariant chains of the TCR complex, which in turn recruit the Syk family kinase Zap70 through its dual SH2 domains (Fig. 3). Zap70 is then phosphorylated and activated by Lck (Lin and Weiss, 2001; Samelson, 2002). Activated Zap70 in turn phosphorylates a number of adaptor molecules, including a novel transmembrane palmitoylated adaptor protein, LAT, which serves as a platform for the recruitment of other signaling molecules. These include GRB-2, which activates the Ras-Raf MAPK pathway, a related molecule, GADs, which binds to the adaptor molecule SH2-domain-containing leukocyte protein of 76 kDa (SLP76) via a proline domain-SH3 interaction, and PLC-γ. SLP76 also serves as a central component of this signaling complex: mutations of either LAT or SLP76 impair TCR activation of ERK and PLC-γ in the Jurkat T cell-line and prevent thymocyte maturation at early stages (Clements et al., 1999). Upon TCR activation, SLP76 is phosphorylated by Zap70 and can bind to the guanine nucleotide exchange factor Vav, the adaptor molecule Nck and the SH2 domain of the Tec family kinase Itk (Bunnell et al., 2000; Clements et al., 1999). Secondary interactions with LAT through the Itk SH3 and SH2 domains and an interaction with PLC-γ also contribute to interactions of the Tec kinases in this complex (Bunnell et al., 2000; Perez-Villar and Kanner, 1999). In B cells, a similar signaling complex is nucleated by the adaptor BLNK/SLP65, which recruits GRB2, PLC-γ, Vav and Btk (Kurosaki and Tsukada, 2000). Reconstitution experiments have demonstrated that Btk function requires both the PH domain and a functional SH2 domain, which bring Btk into this signaling complex (Takata and Kurosaki, 1996).

Within this complex, the Tec kinases appear to be important for full phosphorylation and activation of PLC-γ, an enzyme that cleaves PtdIns(4,5)P_2 to generate Ins(1,4,5)P_3 and diacylglycerol (DAG) (Lewis et al., 2001; Rhee, 2001; Scharenberg and Kinet, 1998). Ins(1,4,5)P_3 then binds to receptors on intracellular organelles to release intracellular Ca^{2+}, which leads to an influx of Ca^{2+} from extracellular sources via store-operated (CRAC) channels (Lewis, 2001). B cells from XLA patients, a mutant DT-40 chicken cell line that lacks Btk, and T cells lacking Itk or Itk and Rlk, all show varying defects in PLC-γ phosphorylation associated with decreased Ins(1,4,5)P_3 production and Ca^{2+} mobilization (Fluckiger et al., 1998; Liu et al., 1998; Schaeffer et al., 1999; Takata and Kurosaki, 1996). In particular, the late phase of Ca^{2+} influx from extracellular stores is most affected in these cells. Although overexpression of Btk and Rlk can increase tyrosine phosphorylation of PLC-γ, whether phosphorylation

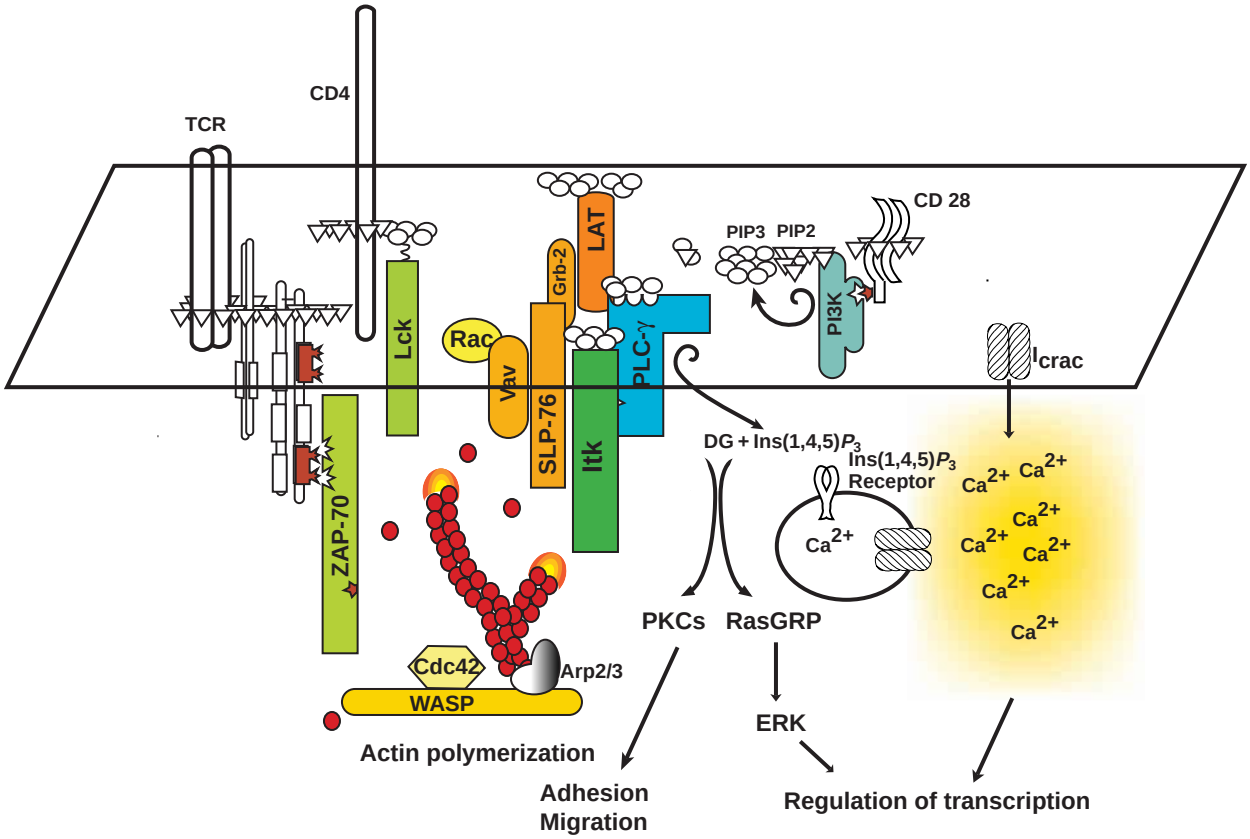


Fig. 3. Involvement of Tec kinases in TCR signaling. The Tec kinases (represented here by Itk) are part of a signaling complex nucleated by LAT and SLP76 that is critical for the activation of PLC-γ, Ca^{2+} mobilization, MAPK activation, as well as for downstream cytoskeletal rearrangements and transcriptional regulation. The red boxes represent phosphorylated ITAMs on the intracellular tails of the invariant TCR chains. Red circles represent actin. PIP2, PtdIns(4,5)P_2; PIP3, PtdIns(3,4,5)P_3.

is the only contribution of Tec kinases to PLC-γ activation and antigen receptor signaling remains unclear (Fluckiger et al., 1998; Veri et al., 2001). Indeed, one study of BTK-deficient DT40 cells showed that kinase-inactive BTK can also improve Ca^{2+} mobilization in these cells (Tomlinson et al., 2001). This observation, along with potential interactions of Tec kinases with PLC-γ and SLP76, suggests additional functions for the Tec kinases (Perez-Villar and Kanner, 1999; Schneider et al., 2000).

Mutation of the Tec kinases is also associated with decreased activation of MAP kinases. In T cells, activation of the MAP kinase ERK requires activation of Ras-GRP (CD-GEFII), a novel Ras-GEF that contains a DAG-binding domain (Ebinu et al., 2000). Decreased activation of ERK has been observed in T cells lacking Itk or Rlk and Itk and may be secondary to decreased production of DAG (Schaeffer et al., 1999). In Btk-deficient B and mast cells, defective activation of the MAP kinases JNK and p38 have also been reported, suggesting other connections to these kinases (Jiang et al., 1998; Kawakami et al., 1997). An adaptor protein RIBP/LAD/TsAd, which can interact with both Rlk and Itk (Rajagopal et al., 1999), also associates with MEKK-2, which activates the MEK-5/ERK5 pathway (Sun et al., 2001).

Note that, unlike mutations of the more proximal tyrosine kinases Lck and Zap70, which eliminate Ca^{2+} mobilization and MAPK activation and severely block thymocyte development, mutation of the Tec kinases in T cells merely reduces the intensity and duration of these signals (Schaeffer et al., 1999). These observations have led to the concept that the Tec kinases have more modulatory roles in antigen receptor signaling pathways. Whether this is caused by redundancy of the Tec kinases or distinct functions for these kinases is unclear. In B cells, regulation of Tec kinases may also contribute to attenuation of B cell signaling through FcγRIIb, which activates SHIP (Scharenberg and Kinet, 1998). Finally, similar signaling complexes exist in mast cells and platelets (Kawakami et al., 1999), although the organization and components vary slightly in each cell, suggesting subtle differences in the requirements for activation and downstream readouts influenced by Tec kinases (Lewis et al., 2001).

Other downstream pathways

Actin cytoskeletal reorganization

Reorganization of the actin cytoskeleton is critical for efficient antigen receptor signaling, during which multiple signaling molecules converge at the plasma membrane to form complexes required for transmitting signals to the nucleus (Acuto and Cantrell, 2000). Moreover, as a signal is transmitted from the exterior, the T cell polarizes and moves into closer contact with the antigen-presenting cell (APC) to form a structure termed the immunological synapse, which may be

required for prolonged TCR signals (Monks et al., 1998). These downstream effects of TCR signaling rely heavily upon movement and rearrangements of an intact actin cytoskeleton. Several emerging lines of evidence suggest that Tec kinases not only regulate PLC-γ but contribute to regulation of actin cytoskeletal rearrangements in response to antigen receptor and other signaling pathways. Indeed, defective actin cytoskeleton reorganization may contribute to the defects in prolonged Ca^{2+} influx observed in Tec-deficient cells (Tsoukas et al., 2001).

The first suggestion that Tec kinases may contribute to actin cytoskeletal regulation came from studies of the *Drosophila* Tec family kinase Tec29 (formerly Src29A), which is required for growth of ring canals (actin-based intracellular bridges between nurse cells and the oocyte). A similar phenotype is observed in mutants lacking the *Drosophila* Src homolog Src64, which interacts with Tec29 and regulates its localization (Guarnieri et al., 1998; Roulier et al., 1998).

In mammalian cells, interactions between the actin cytoskeleton and Tec kinases have been suggested from several experiments. Using a GST-PH domain fusion protein, F-actin was shown to associate directly with basic residues in the N-terminal PH domain of Btk (Yao et al., 1999). Stimulation through the insulin receptor or the G-protein-coupled receptor CXCR4 leads to translocation of a BTK-GFP fusion construct to membrane ruffles or lamellipodia, regions of the plasma membrane formed by actin polymerization (Nore et al., 2000). In platelets, Btk localizes to the cytoskeleton upon activation of the thrombin receptor (Mukhopadhyay et al., 2001). This effect is regulated by the integrin αIIbβ3 and depends on PI3K activity. Furthermore, expression of kinase-inactive Itk reduces TCR-induced actin polymerization in the Jurkat T cell-line (Woods et al., 2001). We have also found that TCR-induced actin polymerization is impaired in Itk- and Rlk/Itk-deficient T cells, supporting the functional importance of these interactions (C. Labno, C. Lewis, J. Burkhardt and P.L.S., unpublished).

Further contributions of the Tec kinases to actin cytoskeleton regulation have been suggested from interactions of Tec kinases with Vav and WASP, two molecules involved in actin reorganization. Vav family members are GEFs that facilitate the exchange of GDP for GTP to activate members of the Rho GTPase family such as Rho, Rac and Cdc42, which are involved in actin cytoskeleton reorganization (Bustelo, 2001). After stimulation with IL-3 and erythropoietin, Vav binds to the Tec kinase via the TH domain of Tec (Machide et al., 1995). A similar complex has been demonstrated with the Prolactin receptor (Kline et al., 2001). When co-expressed in COS-1 cells, Tec was found to associate with Vav and enhance Vav GEF activity. Although these data suggest that Tec kinases participate in the activation of Vav, a recent report argues that Vav may also contribute to the activation of Itk and Tec upon TCR activation (Reynolds et al., 2002), suggesting more complex interactions between these two families of signaling molecules.

WASP (Wiskott-Aldrich syndrome protein) is the gene product responsible for the X-linked hereditary immunodeficiency Wiskott-Aldrich syndrome, a disease associated with defective actin cytoskeleton regulation. Studies have shown that activated CDC42 can bind to WASP, thereby enabling WASP to interact with and activate the Arp2/3 complex, which nucleates new actin filaments (Snapper and Rosen, 1999). A search for Itk SH3 domain ligands revealed that Itk binds to a proline-rich region of WASP (Bunnell et al., 1996). Further studies have demonstrated that Btk can physically interact with WASP and that WASP can serve as a substrate for Btk (Baba et al., 1999; Guinamard et al., 1998). Moreover, we have found defective activation of WASP downstream of the TCR in Itk- and Rlk/Itk-deficient T cells (C. Labno, C. Lewis, P.L.S. and J. Burkhardt, unpublished). The connection between the Tec kinases, WASP and Vav is an intriguing area for future research.

Roles in regulating cellular adhesion

Upon antigen receptor stimulation of T cells, cellular adhesion via integrin receptors is upregulated, a process known as ‘inside-out’ signaling. This effect is absolutely dependent upon the integrity of the actin cytoskeleton. Recently, a novel role for the Tec family kinase Itk was demonstrated in TCR-mediated activation of β1 integrin adhesion (Woods et al., 2001). Increased adhesion required both kinase activity and PI3K-PH-domain-dependent recruitment of the kinase to GEMs. Similarly, we have found defects in TCR-induced adhesion in Itk and Rlk/Itk-deficient T cells (L.F., C. Labno, C. Lewis, J. Burkhardt and P.L.S., unpublished).

Although it is not clear what pathways downstream of Tec kinases help regulate increased cellular adhesion, there are several potential connections. As noted above, Itk can regulate CD3/TCR-induced actin polymerization, which influences downstream adhesion pathways. Another potential connection may involve SLP76 and its binding partner - adhesion and degranulation promoting adaptor protein (ADAP; also known as SLAP-130 or Fyb) - a molecule that has recently been shown to couple the TCR to integrin activation (Griffiths et al., 2001; Peterson et al., 2001). Not only do Tec kinases interact with SLP76, but Rlk can also tyrosine phosphorylate SLP76 in T cells (Schneider et al., 2000). However, whether Tec kinases influence this pathway of integrin regulation remains unclear.

Members of the PKC family of serine/threonine kinases also contribute to integrin activation in lymphocytes, and several lines of evidence suggest that Tec kinases are cross-regulated with PKC isoforms. Studies with the PH domain of Btk reveal that it can interact with multiple PKCs, including PKCβ and PKCξ (Kang et al., 2001; Yao et al., 1994), an isoform that can physically associate with the actin cytoskeleton and is involved in maintaining its integrity (Gomez et al., 1995). More recently, an interaction between Btk and PKCθ was demonstrated in platelets, PKCθ phosphorylating Btk on threonine and Btk subsequently tyrosine phosphorylating PKCθ to downregulate its activity (Crosby and Poole, 2002). PKCθ has also been shown to be a component of the T-cell–APC immunological synapse, a structure that relies heavily upon the actin cytoskeleton (Monks et al., 1998). Therefore, regulation of PKC isoforms may provide a connection between Tec family kinases, cytoskeletal reorganization and integrin activation.

Finally, roles for the Tec kinases downstream of integrin receptors have also been reported. In particular, upon engagement of integrins the FERM domain of FAK can bind Etk/Bmx and activate its kinase (Chen et al., 2001). Moreover, kinase-inactive ETK blocks integrin-mediated migration. Thus, it is possible that integrin function is regulated by Tec kinases at multiple steps.

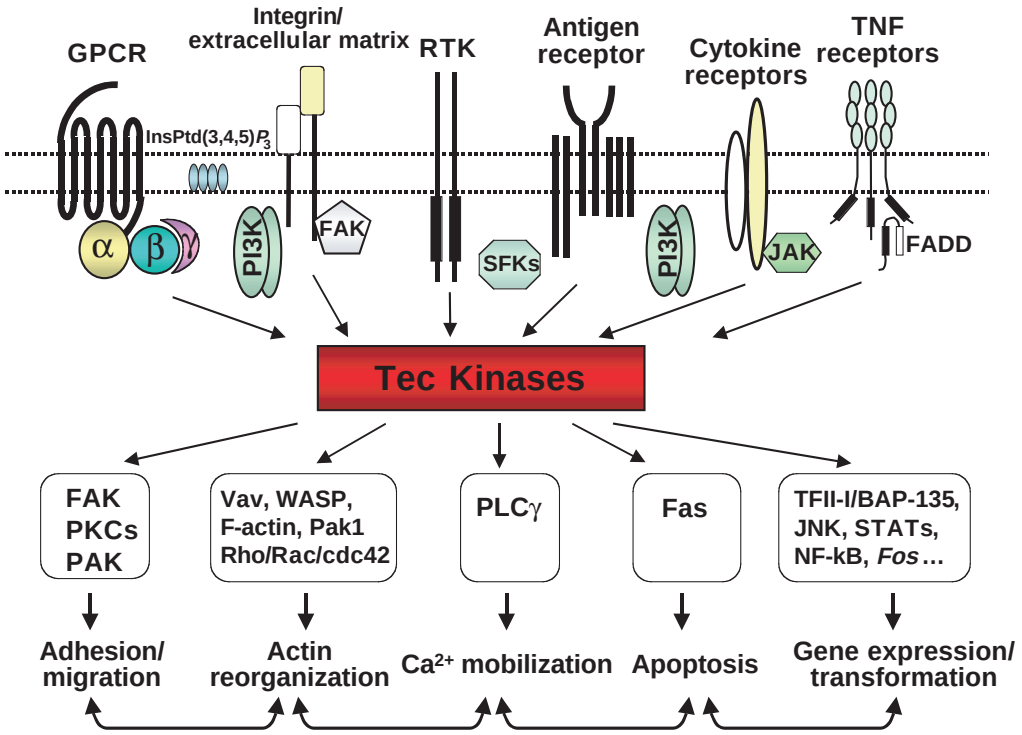


Fig. 4. Signals transmitted by Tec kinases. Genetic and biochemical studies have demonstrated that Tec kinases are activated by multiple membrane receptors and are involved in a variety of downstream responses including Ca^{2+} influx, apoptosis, gene expression, actin reorganization and adhesion/migration. These cell responses may influence each other, suggesting that the Tec kinases can broadly affect cellular physiology.

Transcriptional activation

Mutation of the Tec kinases has also been linked to defective activation of transcription factors. In some cases, these defects can be directly attributed to alterations in upstream activators. For example, mutation of Itk and Rlk in T lymphocytes is associated with decreased activation of the Ca^{2+}-sensitive transcription factor NFAT and the resulting defects in cytokine gene expression (Fowell et al., 1999; Schaeffer et al., 2001). Defective activation of NFKB may be related to defects in Ca^{2+}, as well as DAG-mediated activation of PKC, which is required for activation of this transcription factor (Sun et al., 2000). Similarly, defective AP-1 activation is probably secondary to defective MAPK pathways.

Evidence also supports direct connections between Tec kinases and transcription factors, however. In particular, interactions with and phosphorylation of BAP 135/TFII-I, Bright, STAT3 and STAT5 have been reported (Saharinen et al., 1997; Tsai et al., 2000; Webb et al., 2000; Yang and Desiderio, 1997). Furthermore, it is also now clear that Rlk/Txk, Btk and Itk can all traffic to the nucleus (Debnath et al., 1999; Perez-Villar et al., 2001; Webb et al., 2000). For Rlk/Txk and Btk, this localization may be linked to transcriptional activation. Indeed, nuclear localization may be required for Rlk to induce interferon-γ (IFN-γ) expression in the Jurkat T cell line (Kashiwakura et al., 1999). A recent study extended these findings to show that Rlk directly binds to DNA to stimulate expression of IFN-γ, suggesting novel roles for Tec kinases in the nucleus (Takeba et al., 2002).

Other signaling pathways

Although we have highlighted the roles of Tec kinases in T cell antigen receptor signaling pathways, it is clear that even in lymphocytes, a lack of Tec kinases affects multiple signaling pathways (Fig. 4). For Btk-deficient B cells, these include defects in cytokine (IL-5) and chemokine responses (Kawakami et al., 1999). In T cells, Itk and Tec are activated by the costimulatory molecule CD28, which enhances TCR signaling pathways (August et al., 1994; Michel et al., 2001; Yang et al., 1999). Interestingly, Tec and Itk may have distinct downstream targets in this pathway, highlighting potential diversity in Tec-kinase-mediated signals (Yang et al., 1999). Finally, mutation of the Tec kinases is associated with alterations in cell survival pathways, including regulation of Fas-ligand expression as well as interactions of Fas with downstream molecules required for induction of cell death (Miller and Berg, 2002; Uckun, 1998). Nonetheless, Tec family members appear to have both death-protective and death-promoting activities depending on the system (Uckun, 1998). The complexities of their actions may reflect the multiple downstream effectors these kinases can influence.

In other cell types the Tec kinases have been implicated in additional signaling pathways. The expression of Etk/Bmx in prostate and mammary carcinoma lines suggested a role in cellular transformation. Further studies have revealed that Etk is a critical intermediate in the STAT-3 phosphorylation and activation is required for v-Src transformation (Tsai et al., 2000). Etk/Bmx has also been implicated in G protein signaling pathways in endothelial cells, where activation of Etk by Gα subunits can lead to activation of serum response factor transcriptional elements (Mao et al., 1998). Intriguingly, this pathway appears to involve the small GTPase Rho, an important modulator of the actin cytoskeleton. Etk has also been shown to activate the p21 activated kinase, PAK, another molecule implicated in cytoskeletal rearrangements (Bagheri-Yarmand et al., 2001). Whether other Tec kinases activate similar signaling intermediates remains unclear.

Conclusions

The past few years have revealed that the Tec kinases are important components not only of antigen receptor signaling but also of pathways from multiple cell surface receptors leading to activation of PLC-γ and MAPKs, regulation of the actin cytoskeleton, adhesion, migration and transcriptional activation. As these pathways are further explored, an emerging theme is that the various Tec kinases have both common and distinct requirements for activation, binding partners and substrates. How the Tec kinases contribute to these pathways in various cell types, the interactions between these pathways and the differences between the Tec family members are important questions for the future.

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