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ITK finds another (dance) partner...TFII-I

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Abstract

The signals that regulate T cell activation have been studied for some time. We know that upon interaction with antigen/MHC complex, the T cell Receptor (TCR) triggers the activation of a number of kinases, including tyrosine and serine/threonine kinases. The Tec family kinase ITK plays a role in this response, but the signaling pathways it regulates are less well known. Even less well known are its binding partners and substrates. Sacristan and colleagues have now extended the known partners and substrates of ITK by reporting in this issue of the European Journal of Immunology, that ITK interacts with the transcriptional regulator TFII-I. The implications of this finding are discussed.

Keywords

Tec kinase; TFII-I; T cell signaling

The signals that regulate T cell activation have been studied for some time. We know that upon interaction with antigen/MHC complex, the T cell Receptor (TCR) triggers the activation of a number of kinases, including tyrosine and serine/threonine kinases. The Tec family kinase Interleukin-2 Inducible T cell Kinase (ITK) plays a role in this response, but less well known are the signaling pathways that it regulates. Even less well known are its binding partners and substrates. Sacristan and colleagues have now extended the known partners and substrates of ITK in their report in this issue of the journal that ITK interacts with the transcriptional regulator TFII-I (this issue, [1]).

The Tec kinases was first recognized as a unique family after the discovery of Bruton's Tyrosine Kinase (BTK), mutants of which are responsible for the B-cell immunodeficiency X-linked agammaglobulinaemia (XLA) in humans and X-linked immunodeficiency (XID) in mice [2]. While mice lacking ITK have been described for some time, it was only recently that humans with mutations in ITK were described [3]. ITK deficiency seems to result in a similar phenotype in mice and humans, with reduced numbers of naïve CD4 and CD8 T cells, and a higher frequency of innate memory phenotype or non-conventional T cells, and reduced development and function of *i*NKT cells [4–11]. Compellingly, the ITK deficient patients exhibited Epstein Barr Virus-driven lymphoproliferative disease, also found in patients with other defects in NKT cells, suggesting that ITK plays a critical function in NKT cell responses against this virus [3]. ITK also negatively regulates the development of a unique population of $V\gamma 1.1^+ \gamma\delta$ T cells [12,13]. However, a major function for ITK was originally described by

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Conflict of interest statement

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Fowell and colleagues, showing that T cells lacking ITK have defects in the development and/or secretion of Interleukin-4 [14]. Indeed, genetic analysis in humans has revealed high level expression of ITK T cells from patients with atopic dermatitis and a correlation between a specific ITK SNP haplotype and seasonal allergic rhinitis, both diseases thought to be driven by Th2 responses [15,16]. Finally, ITK null mice do not develop allergic airway disease when challenged in models of allergic asthma [17]. These major effects point to an important role for ITK in regulating T cell activation and function.

Structure and activation of ITK

Like other Tec family kinases, ITK has a Pleckstrin-Homology (PH) domain at the amino-terminus that allows it to reversibly interact with the plasma membrane, most likely in lipid rafts [18]. It also has a Tec-homology (TH) domain, made up of a Zn²⁺ binding domain, and a proline rich region (PRR), and SH3, SH2 and kinase domains round out the structure. These provide ITK with multiple protein:protein interaction domains, and in vitro, ITK can interact with a number of partners, however few have been validated in cells [19].

While the TCR is the most well studied, receptors as varied as CD28, CD2, CXCR4, CXCR3, FcεRI, FCRγ1, NKG2D, and now, as reported by Sacristan et al CD43, can activate ITK [18]. When activated via the TCR, ITK requires Lck, Zap-70 and the presence of LAT and SLP-76 (Fig. 1, [18]). Lck activates phosphatidylinositol 3-kinase (PI3K) downstream of the TCR, generating phosphatidylinositol-3,4,5-triphosphates (PtdIns(3,4,5)P₃, PIP₃). PIP₃ induces the recruitment of ITK to the membrane by direct binding interaction with its PH domain [20]. IP₃ 3-kinase B (ItpkB) also promotes recruitment and regulation of ITK by generating inositol 1,3,4,5-tetrakisphosphate (IP₄), a soluble ligand for ITK's PH domain, promoting its dimerization and enhancing binding to PIP₃ at the membrane [21]. More importantly, Lck is required for the direct phosphorylation and activation of ITK [22]. The requirement for Zap-70 and LAT is not clear, but may reflect Zap-70 phosphorylation of LAT, causing the formation of a multiprotein complex that includes SLP-76, Grb-2 and PLCγ1 [18]. ITK also binds to SLP-76 via its SH2 and SH3 domain, prolonging its activation [18]. ITK also interacts with PLCγ1 and directly phosphorylates it, leading to its activation and generation of IP₃ and DAG, release of intracellular calcium stores, and an increase in intracellular calcium [18]. However, paradoxically, T cells lacking ITK are not deficient in release of intracellular calcium stores, but in influx of calcium from the outside of the cell via Store operated Calcium Channels [23]. Thus ITK regulates the ability of T cells to activate a major transcription factor regulated by calcium, NFAT [14,24]. As NFAT is critical for both T cell development and cytokine secretion, this ability to regulate calcium has been proposed as the major explanation for the effects of ITK on Th2 cytokine production and T cell development, as well as the inability of ITK deficient T cells to produce Interleukin 2 [18]. However, activation of a number of other signaling pathways is reduced in the absence of ITK, including ERK/MAPK, and the transcription factors AP-1 and NFκB [24]. The finding that ITK directly interacts with the transcriptional regulator TFII-I and can induce its tyrosine phosphorylation and function in turning on the c-Fos promoter, suggests other pathways by which ITK may regulate T cell development and function (this issue, [1]).

TFII-I

Originally described by Roy, Roeder and colleagues, TFII-I is an ubiquitously expressed multi-function transcriptional regulator also known as GTF2I, one of several genes implicated in Williams-Beuren syndrome [25–27]. TFII-I has a member of the helix-loop-helix DNA binding domain, a leucine zipper and six I-repeats [25]. These various domains allow TFII-I to not only interact with DNA and regulate gene expression, but also with a large number of binding partners, including BTK, another Tec family kinase [28,29]. TFII-I is phosphorylated on both

serine and tyrosine residues, which affect its ability to regulate transcription, including c-Fos expression via SRF, as well as regulate the transcriptional activity of c-Myc and NFκB [28–31]. Thus this protein controls a number of genes downstream of specific receptors, but while most experiments have examined those downstream of the PDGF receptor or the B cell Receptor (BCR), cytokines that activate JAK2 may also regulate TFII-I, since this kinase can also phosphorylate TFII-I [32]. The new work by Sacristan and colleagues add ITK to this growing list of kinases that can regulate this transcription factor (this issue, [1]).

While a number of TFII-I regulated genes have been identified in other cell types, including those involved in cell cycle progression, it is not clear whether the same genes are regulated in T cells [27,33]. Of significant interest is the finding that TFII-I can regulate expression of HIV-1 in T cells via the LTR [34–37]. Desiderio and colleagues have also shown that in PC12 neuronal cells and HEK293 T cells, TFII-I negatively regulates agonist (UTP and Bradykinin) induced calcium increase [38]. This is due to competition for the SH2 domain of PLCγ, reducing its interaction with Transient Receptor Potential C3 (TRPC3) channels [38]. TFII-I also has other splice forms which have differential functions in transcription [39].

Implications of ITK/TFII-I interaction: a novel signaling pathway or explanations for already known pathways?

So, what are we to make of this new finding of ITK/TFII-I interaction. Similar to what happens when two cultures intersect, this finding opens up novel potential signaling pathways and genes that may be regulated by ITK. A prominent function of TFII-I is the regulation of c-Fos along with SRF [30]. As ITK can also regulate c-fos via SRF, perhaps this ability is controlled by its interaction with TFII-I (Fig. 1, [1,40]). In addition, ITK's ability to regulate SRF is dependent on its SH3 domain, the same domain that Sacristan et al suggest interacts with TFII-I. ITK can also regulate HIV-1 gene expression, and since TFII-I is also involved in this process, it is possible that TFII-I regulates this function of ITK [41]. Both c-Src and BTK can phosphorylate TFII-I, but at different sites, and may thus regulate different aspects of TFII-I function [42, 43]. In addition, Sacristan et al show that ITK also interacts with different domain in TFII-I than those previously shown to interact with BTK [1]. ITK may therefore connect the TCR pathways utilized by BTK and c-Src, but may also activate unique pathways [1]. TFII-I also has other splice forms which have differential transcriptional activity, all of which Sacristan et al show can interact with ITK, suggesting that there may be other aspects of cellular function that are also regulated by ITK via TFII-I [39].

However, most interesting is the previous observation that TFII-I can regulate agonist induced calcium influx into cells [38]. ITK interacts with PLCγ, and regulates TCR induced calcium influx, and it is possible that the ITK/TFII-I interaction has functions beyond direct TFII-I regulated gene expression, but in regulating calcium influx. Perhaps ITK titrates TFII-I away from PLCγ, allowing calcium influx (Fig. 1). Experiments examining TFII-I's role in TCR induced calcium influx may determine if this is the case. Important also is the question of whether there are several pools of ITK in cells that interact with various partners (such as SLP-76) or whether there is one pool of ITK. Like all good work, this finding by Sacristan et al begs more questions, and opens a new avenue for understanding the function of ITK in TCR signaling and function.

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Figure 1.

T cell Receptor induced regulation of ITK, and its interacting partners. The newly identified partner for ITK, TFII-I is indicated in red. Hypothetical interactions are indicated as dashed lines. It is possible that ITK competes with PLCγ1 for TFII-I binding, thus regulating calcium influx. It is also possible that TFII-I regulates the ability of ITK to activate c-Fos.