

Showcase on Research

The Tec Family of Tyrosine Kinases

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A quick survey of the proteins involved in cellular signalling pathways shows that they are composed of a remarkably small set of 'building blocks' or domains. The key to the success of this strategy is that each of these structural units encodes a specific function and in many cases this function involves mediating specific protein-protein interactions. Including multiple copies of these domains in a single protein provides a multi-valence effect, allowing large multi-protein complexes to form. The further advantage of the multi-domain nature of these proteins is that the interaction surfaces are relatively small and modifications such as phosphorylation provide a simple mechanism to encourage or discourage these interactions.

The Tec family of kinases provides an attractive model system to study the roles of modular domains on signalling pathways, as they are each composed of multiple domains, form stable multi-protein complexes, are expressed primarily in hemopoietic tissues and at least one family member is associated with inherited disease. My focus has been on Tec itself, relating the structure of each domain to its function. More recently this focus has shifted to the role of Tec in macrophage differentiation, phagocytosis and cytoskeletal rearrangement.

Domain structure and function

The Tec family of tyrosine kinases includes Tec, Btk, Itk, Bmx and Txk. Each is composed of a C-terminal kinase domain and a number of 'regulatory' domains reminiscent of the structure of the prototypical oncogene Src (Fig. 1). However, two important features of the Src family of proteins, a C-terminal negative regulatory phosphotyrosine residue and N-terminal myristoylation, are absent in the Tec family of proteins (1).

A major defining aspect of the Tec family is the presence of the Pleckstrin homology (PH) domain. Whilst there are many examples of this domain in a range of signalling proteins, including ser/thr kinases, no other tyrosine kinase family contains this domain. The PH domain of Tec is responsible for binding the mem-

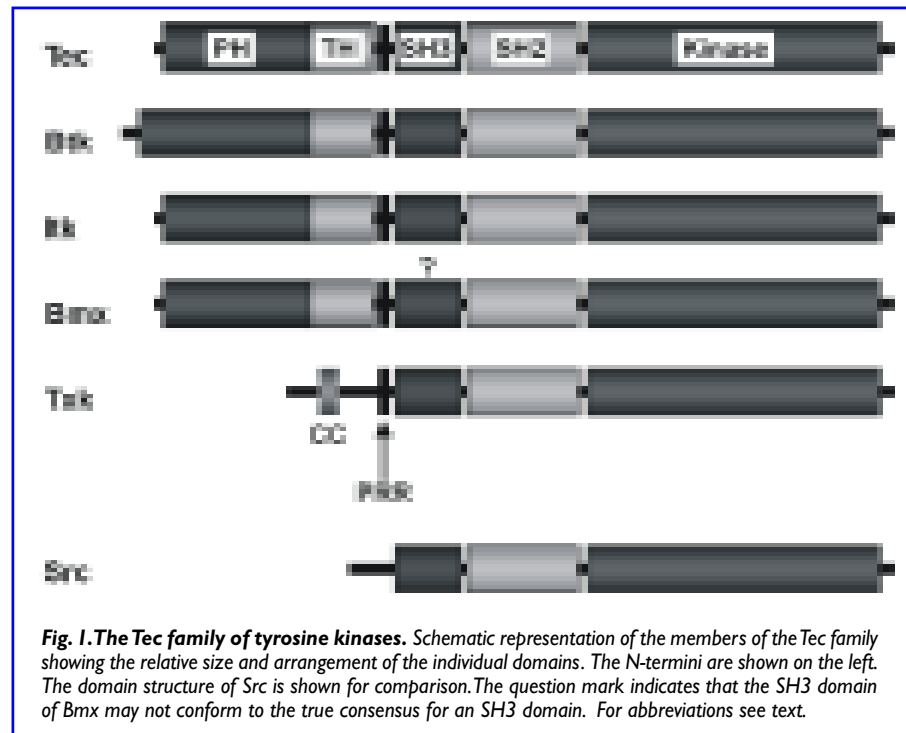


Fig. 1. The Tec family of tyrosine kinases. Schematic representation of the members of the Tec family showing the relative size and arrangement of the individual domains. The N-termini are shown on the left. The domain structure of Src is shown for comparison. The question mark indicates that the SH3 domain of Bmx may not conform to the true consensus for an SH3 domain. For abbreviations see text.

brane lipid second messenger phosphatidylinositol (3,4,5) trisphosphate (PIP3).

The signalling enzyme phosphatidylinositol 3-kinase (PI-3K), itself a multi-subunit, multi-domain enzyme, catalyses the phosphorylation of the 3' position of the inositol ring, thereby generating PIP3. This molecule is seen as an activator in a number of signalling systems and, in the case of Tec, it acts by recruiting the enzyme to the inner surface of the plasma membrane in close proximity to its substrate proteins (2).

The physiological importance of the PH domain was highlighted by observation of the Btk PH domain mutant Arg29Cys in human patients with agammaglobulinaemia (3). This amino acid substitution reduces the affinity of the PH domain for PIP3, leading to a loss of Btk targeting to the membrane and a breakdown in B cell receptor signalling. This loss of B cell receptor function limits B cell development, leading to a lack of circulating mature B cells and, consequentially, the abnormally low levels of serum antibodies associated with disease (3).

The Tec Homology (TH) domain, also

referred to as the Btk motif, consists of a small series of loops held together by a bound zinc ion and appears to be closely linked to the PH domain (4). This physical dependency probably explains why there are many examples of isolated PH domains found in the sequence databases, but the TH domain is only ever found directly C-terminal to a PH domain. The function of the TH domain is not obvious as, apart from the Tec family of enzymes, it has so far only been observed in one class of Ras GTPase activating protein.

The proline-rich region (PRR) links the TH and SH3 domains. This region includes one or two proline consensus sites for SH3 binding. This finding provides the intriguing possibility that intramolecular associations between the PRR and the SH3 domain act to regulate the enzyme's activity. Itk has only one proline motif within its PRR and this has been clearly shown to associate intramolecularly with the Itk SH3 domain (5). Breaking this interaction with a higher affinity SH3 ligand is likely to have an allosteric effect on the enzyme, allowing the active site to become available to substrates in a manner

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analogous to the activation of Src and Hck (6). Deletion of the SH3 domain in Btk has been shown to activate enzyme activity *in vitro*. Interestingly, a splice variant in mouse Tec (Tec III), lacking 22 amino acids from the SH3 domain, appears to be more active and localise differently in cell culture to the 'full length' (Tec IV) version. The biological role of the differential expression of these splice forms is not clear (7).

The SH2 domain of the Tec family of enzymes has been relatively lightly studied. SH2 domains in general, however, are very well understood; they bind phosphorylated tyrosine residues within a sequence specific context. That is, the modified tyrosine is a general prerequisite for binding, but the specificity comes from the SH2 domain surface interacting with the amino acid side chains C-terminal to the target tyrosine (8).

There are two important exceptions to these general comments on Tec family domains. Txk is clearly a member of the Tec family, its gene is immediately 3' of the Tec gene and the majority of the intron/exon boundaries are conserved with Tec and Btk. However, the PH and TH domains are absent in Txk and have been replaced with an example of a cysteine stretch motif.

This region is a signal for palmitoylation and dictates whether Txk localises to the cell membrane or the nucleus (9). The second exception is the SH3 domain of Bmx. This region includes some sequence features of the SH3 consensus, including the Trp-Trp motif, but the match is sufficiently poor to question whether this is a functional SH3 domain. It will be of interest to determine whether this region forms the normal β barrel structure associated with SH3 domains and also whether it binds the proline-rich consensus sequence or some other ligand.

Functional studies *in vivo*

Mouse knockout studies on four of the five family members have shown some interesting results with evidence for functional redundancy (10,11). In the case of Btk and Itk, the knockout phenotype revealed roles in B cell receptor and T cell receptor signalling, respectively.

For Tec and Txk, however, the situation appears more complex. Tec knockout mice display no obvious phenotype even

though this protein is normally expressed in many tissues. When bred with Btk^{-/-} mice, a mild additional B cell defect was revealed, indicating a role for Tec in B cell signalling but one that can be almost fully compensated for by Btk.

A similar story was found for Txk although the degree of overlap with Itk appears to be less. Txk^{-/-} mice display a weak but clear defect in T cell function. When bred with Itk^{-/-} mice, however, there is a dramatic further loss of T cell function, with resistance to lethal infection dropping from 125 days (50% survival) for Txk^{-/-} mice and 69 days for Itk^{-/-} mice to 41 days for the double mutant. The molecular details of these compensatory mechanisms have yet to be defined, although the clear structural differences between Txk and the other proteins suggests that a simple overlap of activities is not likely.

macro-phages and a link to the cytoskeleton through an interaction with the actin cross-linking protein actinin-4.

How these studies are to be reconciled with the quite disappointing phenotype of the Tec^{-/-} mice is not clear, since Ellmeier *et al.* (11) focussed on B cell function and have yet to look for functional defects in the myeloid compartment. Initial evidence from a Tec^{-/-} ES cell model system we have developed indicates that Tec is required for macrophage formation.

Based on the apparent conservation of the B cell, T cell and Fc γ receptor signalling systems, we have proposed the role for Tec in Fc γ receptor signalling shown in Fig. 2. Tec is recruited to the plasma membrane via a PH-PIP3 interaction and is then phosphorylated by a Src family member, probably Lyn. A major downstream target of Tec is likely to be activation of phospholipase C γ , an enzyme

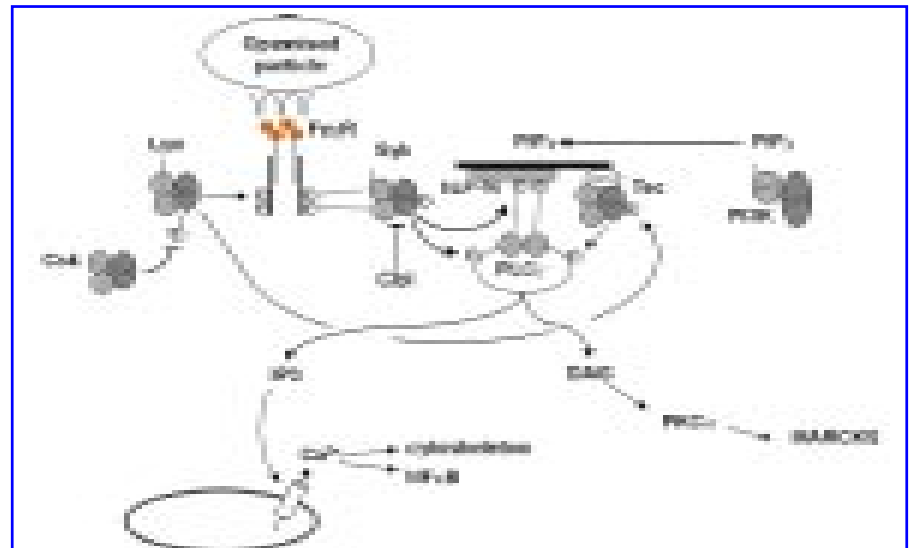


Fig. 2. The role of Tec in Fc γ receptor signalling. Schematic showing the proteins known to play a role in Fc γ receptor signalling, their relative locations within the cell and the binding partners with which they interact. The dotted lines represent SH2-phosphotyrosine interactions.

Functional studies *in vitro*

In contrast to the gene knockout work in mice, a large number of *in vitro* studies has suggested roles for these enzymes in many signalling pathways. For example, Tec has been implicated in signalling pathways for the interleukin (IL)-3 receptor, IL-6 receptor, c-kit, Epo receptor, T-cell receptor, B cell receptor, integrins and some G protein-coupled receptors.

Our own work (see front cover of this issue) has indicated a role for Tec in phagocytosis mediated by the Fc γ receptor in

responsible for cleaving phosphatidyl inositol (4,5) bisphosphate to diacylglycerol (DAG) and inositol (1,4,5) trisphosphate (IP3). IP3 is thought to act on specific ion channels resulting in the sustained Ca²⁺ signalling needed for cytoskeletal rearrangement and cell proliferation. A major question for this pathway remains the link between receptor aggregation and PI-3 kinase activation.

Future directions

It is still relatively early days for the Tec family of tyrosine kinases and there is

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The Role of LIM Proteins in Signal Transduction

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The intricacy and diversity of cell functions require an incredible complexity and specificity of protein interactions (1). LIM domains are one of a growing number of structural motifs implicated in mediating protein-protein interactions required for regulation of transcription and maintenance of the actin cytoskeleton (2). This review will focus on the role of LIM proteins in cell signalling pathways.

LIM is an acronym of three transcription factors *lin-11*, *isl-1* and *mec-3*, in which the motif was first identified. LIM domains contain 50 - 60 amino acids forming a double zinc-finger motif with the consensus sequence (C-X₂-C-X₁₆₋₂₃-H-X₂-C)-X₂-(C-X₂-C-X₁₆₋₂₁-C-X₂-H/D/C) (Fig. 1).

The conserved cysteine, histidine and aspartic acid residues form two tetrahedral zinc-binding pockets, which stabilize the secondary and tertiary structure of the protein. Mutation of either the conserved cysteine or histidine, disrupts both zinc binding and the function of the LIM domain.

The LIM domain is similar to, but distinct from, other zinc-binding motifs designated zinc fingers, which occur in a wide variety of proteins (3). Zinc fingers were initially recognised as DNA-binding mo-

tifs, but have also been shown to bind proteins, mediating dimerisation and interaction with other zinc-finger and non-zinc finger proteins. Evidence to date indicates that LIM domains are protein-binding zinc

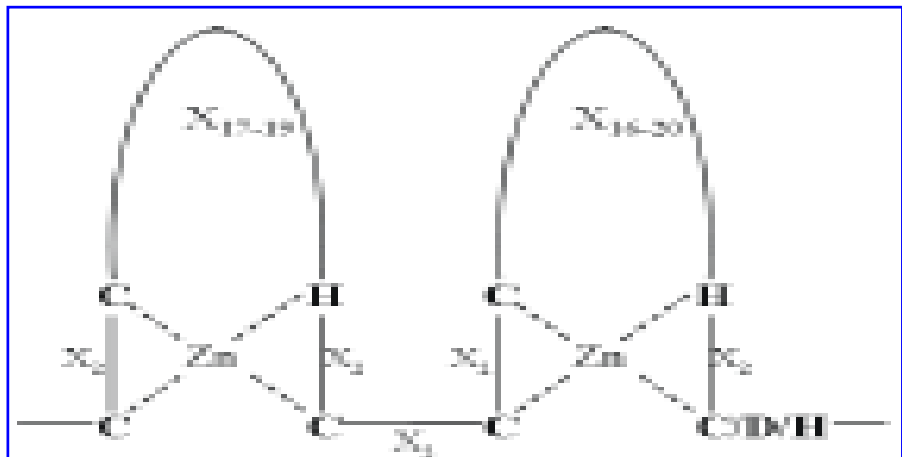


Fig. 1. Schematic representation of a LIM domain. The highly conserved cysteine (C) and histidine (H) amino acids form two zinc-binding pockets. The two zinc fingers are always separated by two amino acids. The more variable sequences of between 16 and 23 amino acids form the two fingers and contain the protein binding sites.

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much to do before we will have a clear understanding of each member's role in signalling, particularly with respect to the possibility of functional redundancy. A key piece of the puzzle is the role of Bmx and the phenotype of the *Bmx*^{-/-} mouse. Another issue is the role for alternative splice forms of these proteins and, at a technical level, the discrepancies between the *in vitro* biochemical studies and the *in vivo* genetic studies in mice.

One may ask, for example: is the myriad of interactions proposed for Tec based on tissue culture experiments mostly artefactual? Alternatively, have we yet to conduct the mice studies in a suitable genetic background or have we looked closely enough for subtle phenotypes? The safest guess, of course, is somewhere in between.

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fingers (4). The presence of two or more LIM domains in most LIM proteins, enables LIM proteins to bind more than one protein, and therefore to act as molecular adaptors or scaffolds for the coordinated interaction of several proteins.

LIM proteins have been divided into three groups according to the amino acid homology between their LIM domains, classified as types A-E (Fig. 2) (2). Group I LIM proteins contain paired N-terminal type A and B LIM domains either alone (Lmo proteins), or in association with a C-terminal homeodomain (LIM-homeodomain proteins) or a kinase domain (LIM-kinase). Group I LIM proteins are predominantly nuclear, with the exception of LIM-kinase I. LIM-homeodomain proteins perform critical roles in embryonic development and tissue differentiation.

Group 2 LIM proteins contain one or two Type C LIM domains followed by a glycine-rich region with no other significant signalling domains. They include cysteine-rich protein 3/ muscle LIM protein (CRP3/MLP), targeted deletion of which has been shown to cause a dilated

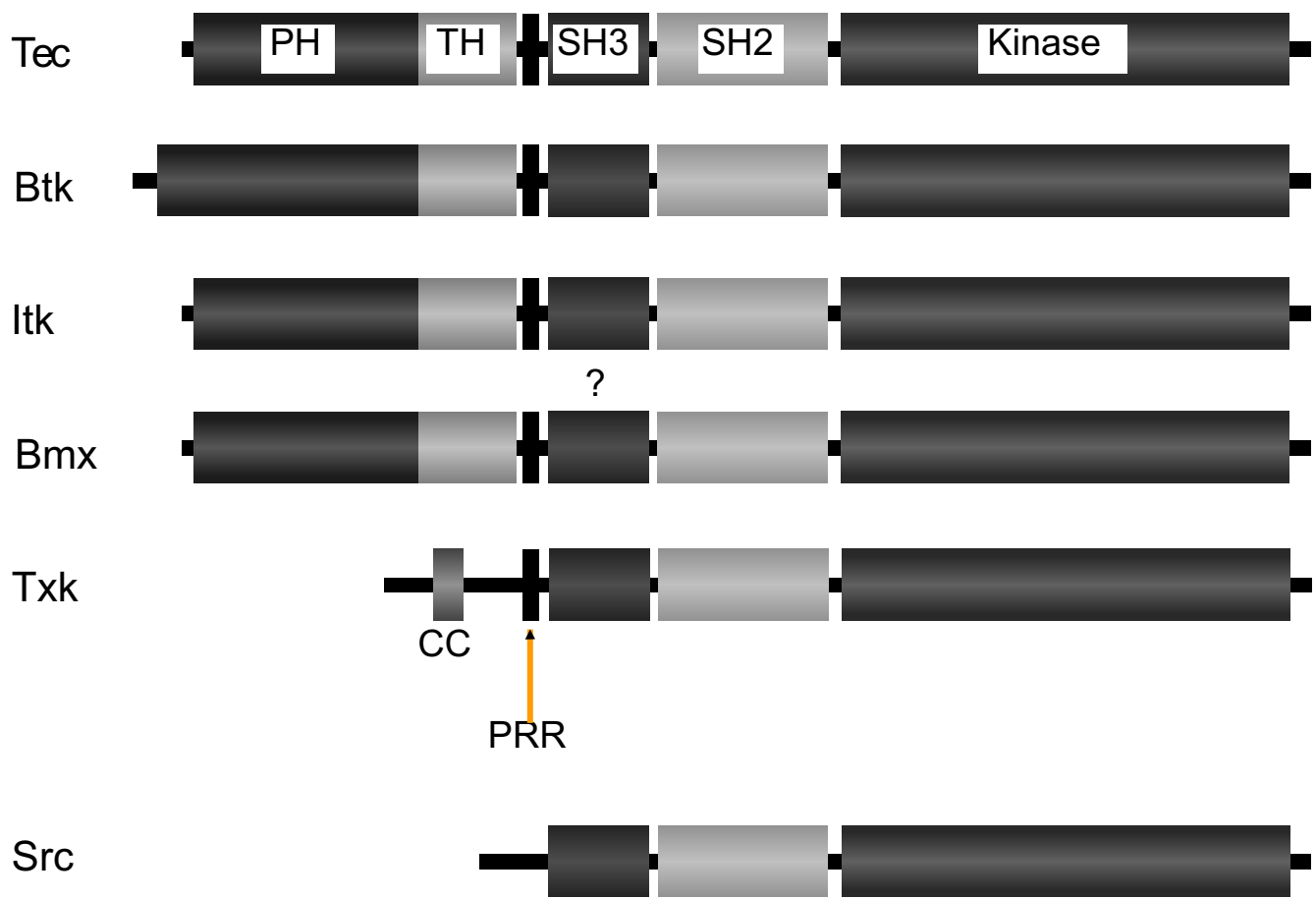


Fig. 1

