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BTK, THE TYROSINE KINASE AFFECTED IN X-LINKED AGAMMAGLOBULINEMIA

Mauno Vihinen[1], Pekka T. Mattsson[2,3] and C. I. Edvard Smith[2]

[1]Department of Biosciences, Division of Biochemistry, P. O. Box 56, FIN-00014 University of Helsinki, Finland
[2]Center for BioTechnology, Department of Bioscience at Novum, Karolinska Institute, S-141 57 Huddinge and Department of Immunology, Microbiology, Pathology and Infectious Diseases (IMPI), Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden
[3]Department of Biochemistry and Food Chemistry, University of Turku, Vatselankatu 2, Arcanum, FIN-20014 Turku, Finland

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1. ABSTRACT

X-linked agammaglobulinemia (XLA) is a heritable immunodeficiency disorder that is caused by a differentiation block leading to almost complete absence of B lymphocytes and plasma cells. The affected protein is a cytoplasmic protein tyrosine kinase, Bruton's agammaglobulinemia tyrosine kinase (Btk). Btk along with Tec, Itk and Bmx belong to a distinct family of protein kinases. These proteins contain five regions; PH, TH, SH3, SH2 and kinase domains. Mutations causing XLA may affect any of these domains. About 200 unique mutations have been identified and are collected in a mutation database, BTKbase. Here, we describle, the structure, function, and interactions of the affected signaling molecules in atomic detail.

2. INTRODUCTION

X-linked agammaglobulinemia (XLA) is a human immunodeficiency disorder which is caused by a B lymphocyte differentiation arrest affecting the transition of B cell progenitors into mature B lymphocytes. The disease afflicts about 1/200,000 individuals (1). XLA is frequently recognized as the prototype of primary immunodeficiency (PID) (2) and was the first human immune disorder in which an underlying defect - the absence of gammaglobulins - was clearly identified (3). XLA is characterized by an increased susceptibility to infections, mainly those caused by extracellular bacteria (1, 4). In affected individuals, enteroviral infections frequently run a severe course and often resist therapy (1, 4, 5). Using two different approaches, the gene affected in XLA was simultaneously isolated by two groups and found to encode a novel cytoplasmic (non-receptor) tyrosine kinase designated *B*ruton's agammaglobulinemia *t*yrosine *k*inase, Btk (6, 7).

As reviewed in great detail (1, 8), the analysis of serum using electrophoresis, which had only recently been applied in a clinical setting, revealed the absence of detectable immunoglobulins and prompted Bruton to initiate substitution therapy with gammaglobulins.

The increased susceptibility, mainly to bacterial infections in XLA, most often begins during the first year of life when the transferred maternal Ig has been catabolized. There is a pronounced decrease in Ig levels of all isotypes and a virtual absence of humoral response to recall antigens. B lymphocyte and plasma cell numbers are decreased, whereas T lymphocyte subsets are normal and may show a relative increase. The defect is caused by a differentiation arrest confined to the B cell lineage

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1 To whom correspondence should be addressed, at Department of Biosciences, Division of Biochemistry, PO Box 56, FIN-00014 University of Helsinki, Finland. Tel #: +358-9-708 59081, Fax #: +358-9-708 59068. Email: mauno.vihinen@helsinki.fi

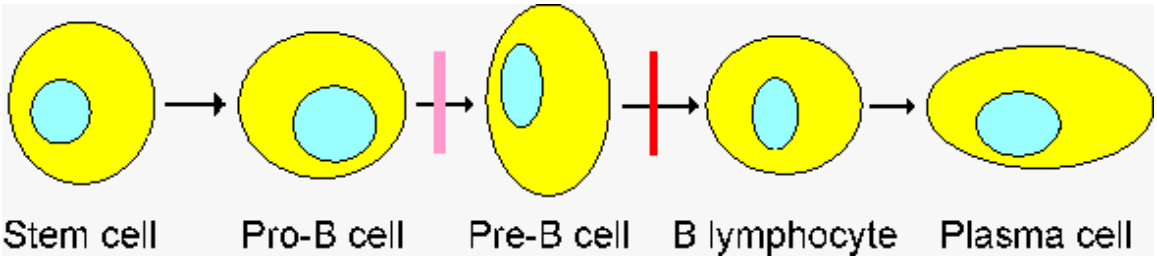

Figure 1. Schematic representation of B lymphocyte differentiation pathway and the differentiation block/growth arrest in XLA. The pink line represents a partial block and the red line an almost total block in B lineage differentiation in XLA.

(Fig. 1), distinguishing XLA from several other Ig deficiencies. B lineage cells in all organs are affected resulting in a reduced size of secondary lymphoid organs such as lymph nodes and tonsils.

3. SPECTRUM OF INFECTIONS AND TREATMENT

The onset of symptoms varies extensively; most patients will show an increased frequency of infections during their first year of life, whereas a few may be asymptomatic until adolescence. Pneumonia, otitis media, and diarrhea are frequent clinical presentations. Sinusitis, conjunctivitis, and pyoderma are also prevalent. Spread of the infection through the blood results in septicemia, meningitis, septic arthritis and sometimes osteomyelitis. Thus, a highly increased frequency of infections is seen essentially in all organs, with the possible exception of the urinary tract, in which only infections with mycoplasma species seem to be overrepresented (4, 9).

Typically, in patients with XLA the infections are bacterial and are caused by *Haemophilus influenzae* or *Streptococcus pneumoniae*. These infections affect all individuals with defective humoral immune responses. However, in contrast to most other primary humoral immunodeficiencies, enteroviral infections may cause an often fatal, slowly progressing disease affecting the central nervous system (5, 9). Thus, antibodies directed against these viruses play a pivotal role in the immune defense.

Bacterial infections are treated with a high dose of antibiotics for prolonged periods. The enteroviral infections may respond to gammaglobulins, but this is not always the case. However, it seems as if high dose gammaglobulin prophylaxis prevents enteroviral infections (9). High dose gammaglobulin given by intravenous or subcutaneous infusions also decrease the number of bacterial infections.

4. THE XLA GENE ENCODES A TYROSINE KINASE

In the 1980s the gene defective in XLA was mapped to the Xq21.3-22 region in the mid-portion of the long arm of the X-chromosome. In particular, the marker DXS178 proved to be useful as it segregated with the disease gene in all families analyzed (10, 11). This marker was also crucial in the positional cloning of the XLA gene as it was used in the selection of yeast artificial chromosomes which later was employed in the enrichment of cDNAs from B lineage cell lines (6). Two missense mutations affecting critical regions in the kinase domain as well as gross deletions in the gene demonstrated that the isolated gene in fact encoded the disease gene (6). In a search for novel tyrosine kinases, a new gene was found to map to the X chromosomal region implicated in XLA (7). These authors demonstrated the absence of mRNA as well as the corresponding protein in some patients, thus strongly implicating this gene as the gene defective in XLA. Although initially different names were employed in the two cloning papers, it was later agreed to use a common name, Btk.

BTK was found to encode a cytoplasmic protein-tyrosine kinase (PTK). These kinases are sometimes called non-receptor PTKs to distinguish them from the membrane-bound receptor PTKs such as platelet-derived growth factor receptor (PDGFR). Btk resembles Src, but forms a distinct family together with Tec and Itk (Table 1). A fourth member was later isolated from human bone marrow and was designated Bmx (12). It has been suggested that Txk (13, 14) also belongs to this family. These proteins are called the Tec family, as Tec was the first kinase of this family to be isolated (15). The family has the following characteristics:

(i) in the N-terminus, there is a region designated pleckstrin homology (PH) domain. This region is believed to have a membrane-localizing function. In the Src family myristylation in the N-terminus serves this function, but the Tec family kinases lack a myristylation consensus sequence.

(ii) the PH domain is followed by the TH region, which is unique to the Tec family.

(iii) Between the C-terminus and the TH region there are three domains, SH (Src homology) 3, 2 and 1, showing the same order as in Src (Fig. 2). The SH2 and SH3 domains have binding functions, whereas

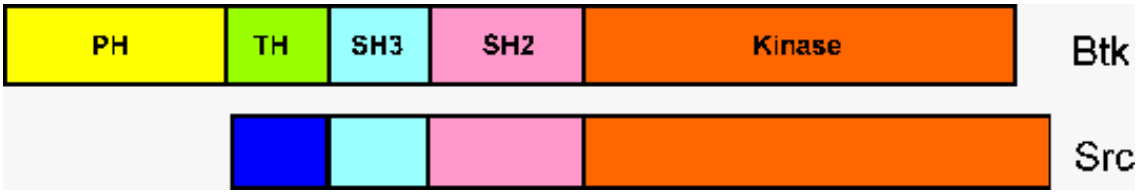

Figure 2. Domain organization of Btk and Src family of kinases.

the SH1 (kinase) domain is catalytic which enables these enzymes to phosphorylate tyrosine residue(s). In contrast to Src family members, but similar to other non-Src kinases, Btk lacks a regulatory tyrosine residue in the C-terminus.

5. ACTIVATION OF BTK AND ITS CONNECTION TO SIGNAL TRANSDUCTION PATHWAYS

The Tec family of proteins appears to be involved in a vast array of signal transduction pathways. The signaling inadequancies in Xid mice suggest a pivotal role for Btk in lympho-hematopoietic growth and differentiation (16-21). The characteristics of the Tec family of PTKs are summarized in Table 1. Except for Bmx, which is expressed in bone marrow and endothelial cells, the members of the Tec family are mainly expressed in hematopoietic cell lineages. All Tec family kinases share the same organization consisting of PH, TH, SH3, SH2, and SH1 domains. The multiplicity of signals is guaranteed by different specificities and interplay of the domains of various proteins. A critical role for these domains in Btk associated signal transduction has been demonstrated by mutations found in XLA patients (28-31).

The crucial role of Btk in B cell differentiation has been studied by searching molecules regulating the activity of Btk and connecting it to various signal transduction pathways (32, 33). In Table 2, the known interactions of Btk and the regulation of Btk activity in B and mast cell signaling pathways are summarized. A gain-of-function mutation (Btk*) capable of transforming NIH3T3 cells has a single point mutation, E41K, in the PH domain (51). The Btk* exhibits increased tyrosine phosphorylation and a 5-fold enhancement in the membrane targeting (51). Although there is no evidence for direct interaction with $G_{\beta\gamma}$ subunits or activation through the PH domain, Btk activity is stimulated by co-transfection with the subunits of heterotrimeric G protein (50).

Several PH domains have been found to associate with different phosphoinositides (56-65). It is possible that some PH domains serve as membrane-binding/associating units. Recently, PH domain of human βΙΣΙΙ spectrin was localized to the plasma membrane *in vivo* (66). Dbl PH domains may mediate cellular targeting to specific cytoskeletal locations (67). According to activation studies, also Btk is predominantly membrane-associated in cells (38). In additon, Btk PH domain interacts with both Ca^{2+}-dependent and Ca^{2+}-independent isoforms of protein kinase C (PKC) in mast cells resulting in inhibition of Btk (49). The cross-linking of IgE receptor (FcεRI) has been shown to result in the activation of Btk (44). As the PKC plays important

Table 1. A summary of the Tec family PTK characteristics. Alternative or previously used names are in parenthesis.

Tec family member	Btk (Atk, Bpk, Emb)		Tec		Itk (Tsk, Emt)		Bmx
Origin of abbreviation	Bruton's tyrosine kinase		Tyrosine kinase expressed in hepatocellular carcinoma		IL-2 inducible T-cell kinase		Bone marrow kinase gene on the X chromosome
Cell distribution	Hematopoietic cells, not in T or plasma cells		T lymphocytes and myeloid cells		T lymphocytes and mast cells		Bone marrow, endothelial cells
Species	Human	Mouse	Human TecI	Mouse	Human	Mouse	Human
Chromosomal location	Xq22	X	4p12	5	5q31-32	11	Xp22.2
Size (aa)	659	659	631	630	620	625	675
MW (kDa)	77	77	74	74	72	72	80
GenBank accession no.	X58957	L29788	D29767	S53716	D13720	L00619	X83107
Refs	6	22	23	24	25	26	12

Table 2. The known connections of Btk and its regulation in mast cell and B cell signaling pathways.

SIGNALING PATHWAY INITIATOR OR MEDIATOR	SPECIES/ CELL TYPE	RESPONSE IN SIGNALLING PATHWAY	REF
B-cell receptor (sIgM)	Mouse WEHI231 cells Human Ramos cells	Increase of kinase activity and phosphorylation of Btk. Temporal activation of PTKs (Lyn/Blk>Btk>Syk)	34-36
Src family kinases: Src, Blk, Fyn, Lyn, Hck	Rat-2 cells, COS-7 cells, NIH 3T3 cells	Trans- and autophosphorylation of Btk at Y551 and Y223, respectively; The data suggest Src family kinases function upstream of Btk. Btk TH domain PRR binds to Fyn, Lyn, and Hck SH3 domains.	36-43
IgE receptor (FcεRI) cross-linking	Mouse mast cells	Activation and phosphorylation of Tyr, Ser and Thr residues of Btk induced by FcεRI cross-linking	44
Activation signals: thymus-independent type 2 antigens, Ig cross-linking and IL-5, IL-10, CD38, CD40 / B7-1 (CD80) and B7-2 (CD86) stimulation	*Xid* mouse (CBA/N) B cells	Abnormal response of *Xid* B cells to activation signals e.g.; arrest in B cell proliferation upon ligation of CD 40, unresponsiveness of B cells to CD38 stimulation with growth co-factors and impairment in the induction of the physiological ligands of CD28, B7-1 and B7-2. Apoptotic cell death of Xid B cells after stimulation of sIgM.	16-21, 45, 46
IL-5	Y16 mouse B cells	Btk activation by IL-5 stimulation	47
IL-6 / soluble IL-6 receptor	mouse BAFBO3 B cells	Activation of Btk and Tec induced by stimulation of gp130	48
Protein kinase C (PKC)	Mouse mast cells	Inhibition of Btk activity by interaction of PKC with Btk PH domain	49
G protein βγ subunit	HEK 293 cells	Btk and Itk activities are stimulated by certain subunits	50
Btk containing a PH domain with a E 41K mutation (Btk*)	NIH 3T3 cells	The Btk* shows transforming activity and an increase in Tyr-phosphorylation and membrane targeting	51
Btk	DT40 B cells	PH and SH2 domains of Btk are needed for PLC-2 activation	52
Btk	DT40 B cells	Btk acts as mediator in radiation-induced apoptosis; kinase domain is essential for the apoptotic response	53
Btk SH3 domain	Burkitt's B cells (Daudi)	Btk SH3 domain binds c-*cbl* protooncogene product $p120^{cbl}$ *in vitro*	54
Hapten (4-hydroxy-3-nitrophenyl)acetyl	*Xid* mouse (CBA/N)	Reduced serologic primary immune response of *Xid* mice leads into a decreased generation of antibody forming cells	55

roles in many signal transduction pathways, including the FcεRI signaling pathway (68), these results together with the membrane translocation of Btk and the activation of PLC-γ (52) suggest Btk to function in membrane-proximal events following FcεRI cross-linking (44).

The stimulation of antigen-specific B cell receptor (BCR) is intimately linked to the activation of three cytoplasmic tyrosine kinase families, namely the Src family, the Tec family and the Syk family (32). Time course -studies implicate temporal activation of these proteins. Src family kinases are activated first (5-10 seconds). This is followed by activation of Btk (2-5 minutes) and then Syk family

of kinases (10-60 minutes) (36). This indicates a downstream role for Btk and Syk kinases in the signaling pathway which is initiated by the Src kinases. Recently, Btk activation was shown to correlate with the dose of Src family kinase activity (40).

The mechanism by which the Src kinases regulate Btk activity is not known in detail. The Blk, Fyn, Lyn and Hck may regulate Btk through an indirect mechanism, in which autophosphorylation of Btk Y551 is required for Btk activity (37). This observation is further supported by the interaction of Btk TH domain and Src family SH3 domains (43). In another study, Lyn was shown to activate Btk by transphosphorylating Y551 in the activation loop, after which Btk autophosphorylates at Y223 in the SH3 domain (38, 39), presumably affecting interactions with its partners. The identity of the partners of Btk during its activation and B cell differentiation is not yet known.

A number of activating signals lead to an increase in Btk activity and tyrosine phosphorylation. Btk and Tec are both stimulated via gp130 a receptor component of the interleukin-6 (IL-6) family of cytokines (48). Both kinases associate with gp130 in the absence of ligand (48), although the nature of the interacting domains remains to be determined. The growth factor IL-5 induces proliferation and differentiation of B cells by binding to receptor IL-5R and leads to the tyrosine phosphorylation of cellular proteins. IL-5 activation also stimulates JAK2 and Btk kinases (47).

Btk is involved in radiation-induced apoptosis in DT-40 lymphoma B cells. Btk, but not Lyn, Syk or Csk, mediates the radiation-induced apoptosis in a kinase domain-dependent manner (53). Recently, Xid B cells stimulated through surface IgM but not CD40 were shown to undergo apoptotic cell death (46). Cell viability correlates with the expression of bcl-x_L, a molecule which blocks apoptosis. bcl-x_L is suggested to be the first inducible protein downstream of Btk (46).

In Xid mice, serologic primary immune response is reduced due to substantially decreased number of memory B cells (55). The magnitude of the secondary response is not limited indicating that the reduced memory B cell number still exeeds a threshold value necessary for a normal secondary immune response (55).

6. STRUCTURAL CONSEQUENCES OF BTK MUTATIONS

The structure of four of the five Btk domains has been modeled to study structure-function and genotype-phenotype interactions (69-72). The gene defect leading to XLA has been characteized in a large number of patients. The mutations have been collected into a database called BTKbase (28-31). Recent analysis of the registry indicated that in the 368 XLA patients, in 318 unrelated families, mutations are scattered throughout the entire length of *BTK* gene (31). The proportion of unique mutations is 72% (228 cases), and the distribution of the mutations in the five structural domains corresponds to the length of the domains. Exonic mutations are distributed as follows: 123 families had missense mutations, 66 had nonsense mutations, 24 showed insertions, and 57 had deletions. In addition, there are 49 intron mutations affecting splice sites. Three double mutations and a single triple mutation have been detected. The gene defect of nine gross deletions have not been characterized in detail. As expected, the missense mutations appear mainly in the first two positions within the codon. Altogether, there were in the missense and nonsense mutations 135 transitions and 54 transversions corresponding to 71 and 29% of the single amino acid substitutions, respectively. Eight of 18 CpG containing arginine residues were affected, whereas none of the residual 15 CpG sites encoding non-arginine residues were mutated. CpG dinucleotides are involved in all the cases where at least five families have the same mutation except for the initiation site. The larger deletions encompass whole exons.

The models of the domains have been used to give putative structural description for each of the XLA mutations. The BTKbase is available at World Wide Web at:
[http://www.helsinki.fi/science/signal/btkbase.html].

6.1. PH domain

Highly divergent pleckstrin homology (PH) domains of about 120 residues have been found in a number of signaling and cytoskeletal proteins including protein kinases and their substrates, phospholipase C, GTPase activating proteins, guanine nucleotide releasing factors, and adaptor proteins (73-79). The Tec family kinases are the only PTKs which contain a PH domain. The 3D structure has been determined for several PH domains. Although these proteins share very limited sequence identity, they have the same fold consisting of a β-barrel formed of two β-sheets and a C-terminal α-helix that caps one end of the β-barrel. The Btk PH domain was modeled (72) based on the dynamin structure (80, 81).

The N-terminal half of at least certain PH domains bind phosphoinositides and the binding residues have been localized in the pleckstrin and spectrin PH domains (57, 59, 63). Site-directed mutagenesis of the three conserved lysines in a charged patch of the pleckstrin PH domain significantly decreased the binding (61). The binding is specific to PIP_2, having a K_d of 41 μM (57).

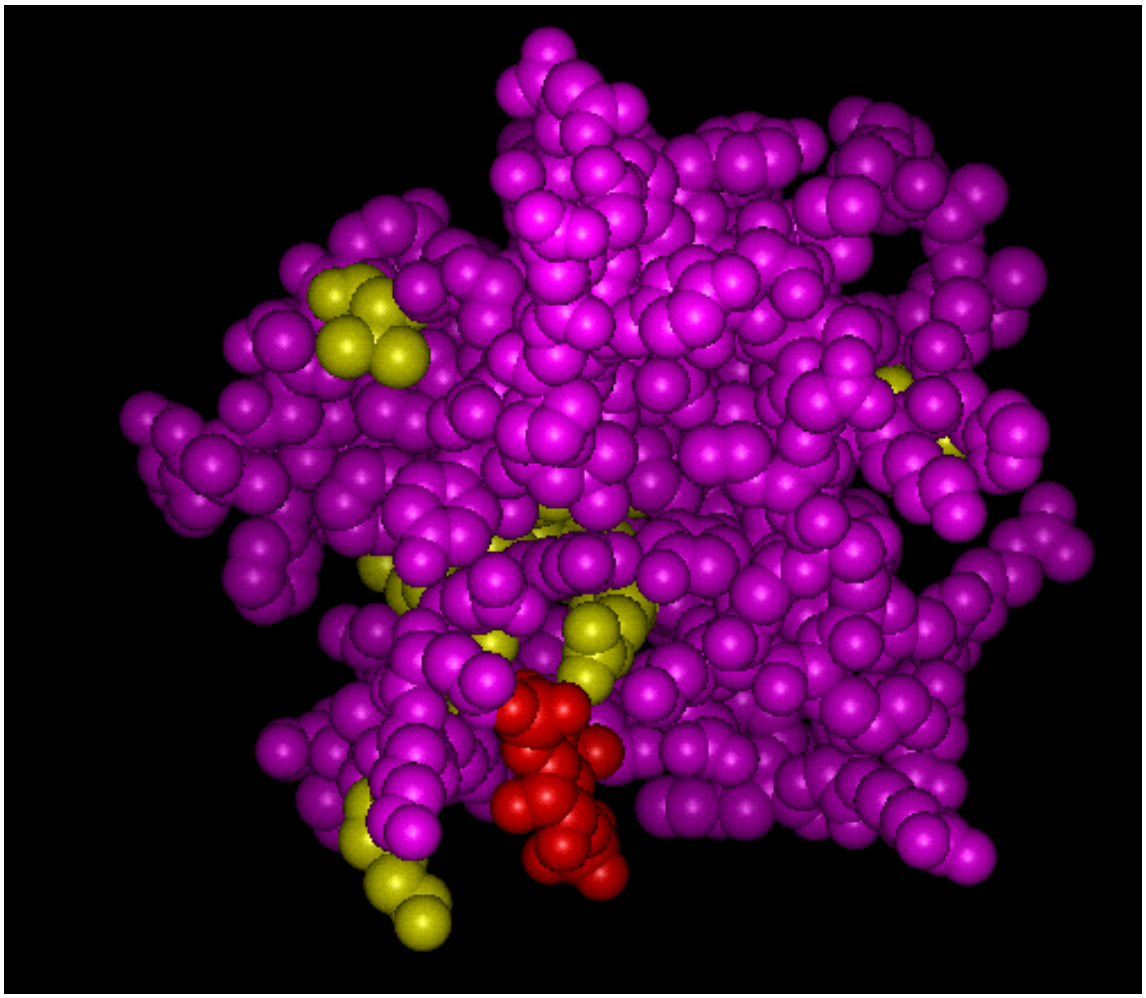

Figure 3. The modeled structure of Btk PH domain with bound PIP_3 (red) in the same site as in pleckstrin and spectrin. The PH domain is displayed as surface presentations. Residues affected by XLA-causing mutations are shown in yellow.

In view of clustering of XLA mutations, the corresponding region in Btk PH domain initially was thought to be involved in binding (72). It was later shown that mutations of Btk residues close to the sites corresponding to the conserved lysines led to the disease (72). According to biosensor assays, Btk PH domain specifically binds to unilamellar liposomes containing PIP_3 in a R28 dependent manner with a K_d of 1.23 μM (82). A point mutation in the PH domain has been shown to cause X-linked immunodeficiency (Xid) in mice (R28C) (83, 84) and XLA in man (31). The modeled Btk structure indicated presence of a putative binding site that could consist of two parts; a highly charged patch and a cleft formed by hydrophobic and aromatic residues (72). The PH domain has been suggested to replace the function of myristylation in membrane targeting of at least some cytoplasmic proteins. Recently, substitution of Btk PH domain residue, E41, by lysine was shown to increase phosphorylation of tyrosine residues and membrane targeting (51). Thus, the Btk phosphorylation might be linked to membrane interaction. Most of the Btk PH domain mutations are concentrated in the binding site region where they could disturb interactions (Fig. 3). The other mutations usually distort the folding of the domain.

Many PH domains, including Btk, have been shown to bind to βγ subunits of heterotrimeric G proteins (85, 86). Only the C-terminal half of the PH domain and some 30 residues from the following TH domain are required for this interaction (85). Btk and Itk kinase activity is stimulated by $G_{\beta\gamma}$ subunits and some unidentified membrane factor(s) (50).

6.2. TH domain

The Tec family members contain a unique region between the PH and SH3 domains which is tentatively designated the TH (Tec homology) domain (87, 88). Conserved N-terminal Btk motif is followed

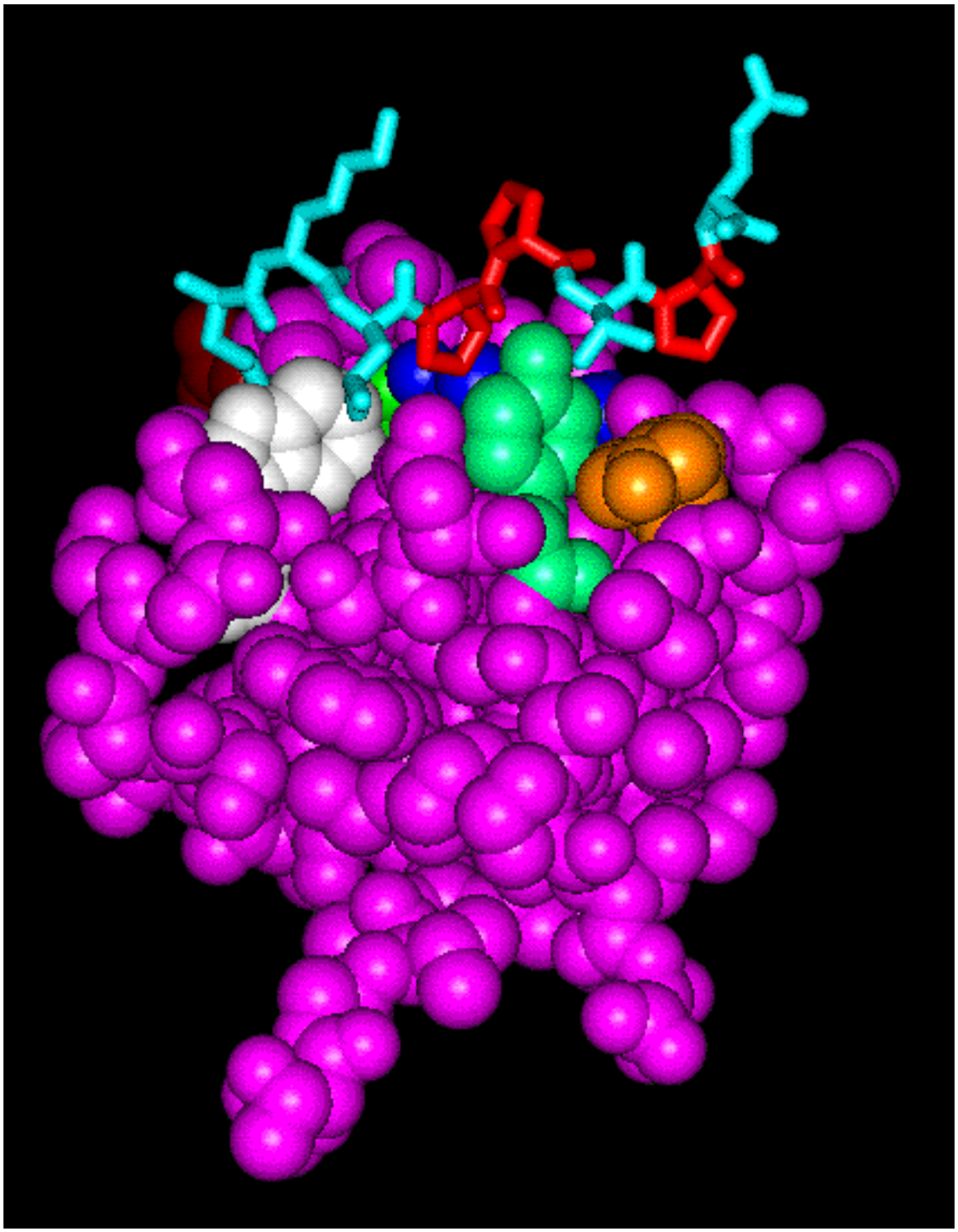

Figure 4. The first proline rich repeat of Btk TH domain docked into Fyn SH3 domain (90). The binding residues in the Fyn are color coded as follows: Y91 orange, Y93 blue, D100 green, W119 white, and Y137 turquoise. In the Btk PRR peptide (cyan), proline residues are red.

by a proline rich region (PRR). Although the whole TH domain can be found only in the Tec family members, the PH domain followed by the Btk motif is present also in several forms of Ras GTPase-activating protein 1 (Ras-GAP1) and in a putative interferon-γ-binding protein (88). The Btk motif contains invariant histidine and cysteines residues which in many cases are involved in metal binding.

Two 10 amino acid motifs in the PRR of Btk have been shown to interact with the SH3 domains of Fyn, Lyn and Hck (41-43), but no data are yet available for association of full length Src family kinases. Itk PRR is also bound by the same proteins with the same specificity (41). The corresponding region of Tec binds to Lyn (89). The Src family kinases are activated early in the B cell activation.

Erythropoietin and IL3 stimulation induces the specific binding of Vav to Tec through the TH domain (90). An unidentified, 72 kDa protein, binds to residues 186-192 in the TH domain of Btk suggesting this domain to mediate stable protein-protein interactions (43).

Of the TH domain binding Src family SH3 domains (41, 43), the 3D structure has been determined for Fyn (91-93). The first of the Btk proline rich repeats was modeled based on the high-affinity peptide binding to c-Src (94) and docked into the SH3 domains (Fig. 4), because the Src family kinases have been shown to preferentially bind this region (43). The binding of the Btk PRR peptide is similar to the other known high affinity interactions. The TH domain PRRs have RLP type sequences (94).

Compared to Src for which high-affinity peptide complex structure is available (94), the polyproline binding residues in Fyn (Y91, Y93, D100, W119, and Y137) are identical. The positions of these residues are also similar, but still the binding specificities are different (42, 95, 96). Subtle changes are known to alter affinity and specificity of SH3 domains (97-99).

Site-directed mutations of the polyproline II (PPII) helix forming proline residues in the PRRs of Btk abolish binding to SH3 domain (41, 43). Mutations, P189A and P192A (41, 43), are likely to alter the conformation such that the polyproline stretch can no longer be recognized. Also, mutation in the conserved polyproline binding region of Fyn (W119L) abolished the binding (43). On the other hand, mutation in another PPII binding site amino acid, D100N, had little or no effect on binding (43).

The Btk PRR does not bind to SH3 domains from Abl, Blk, Btk or Crk (43). The structure of the Btk SH3 domain has been modeled (69). Although Btk SH3 domain has not been shown to bind to the TH domain (41, 43) the modeled PRR peptide was also docked into the SH3 domain and the binding was compared to the Src family binding. The binding site is very similar to that of Fyn and Src. All the major binding residues are conserved (Y223, Y225, D232, W251, and Y268) and have corresponding positions. Although, binding by these residues may occur, other interactions outside this region are likely to be crucial. It is known that Hck affinity is more than 300 fold higher for a full length Nef protein compared to synthesized peptide motif (99).

6.3. SH3 domain

The SH3 domains are modules which bind polyproline stretches containing polypeptides and proteins. The Btk SH3 domain was modeled based on the Fyn structure (69). The Btk SH3 domain has been shown to interact with the proline-rich c-*cbl* protooncogene (54).

There are several nonsense mutations in the Btk SH3 domain, but no known missense mutations have been found (30, 31). Aberrant splicing and skipping of exon 9 leads to an in-frame deletion of 21 residues containing the 14 C-terminal residues of the SH3 domain in two unrelated families (69, 100). Even though this protein is expressed in a stable form in cells and has full kinase activity *in vitro*, the patients have classical XLA (69). Deletion of the last three β-strands seems to distort the structure. According to molecular dynamics simulation, the mutant protein has a stable structure. The spacing between the termini in the mutant protein corresponds to the normal Btk SH3 domain thus facilitating connection to the rest of the Btk without major changes in the overall scaffolding (69).

6.4. SH2 domain

SH2 domains bind phosphotyrosine (pY) containing peptides and proteins. The specificity is gained by recognizing residues from C-terminal to pY. The Btk SH2 was modeled using v-Src as a template (71). The two β-sheets and the terminal α-helices are conserved. Most of the XLA-causing Btk SH2 domain mutations disrupt the pY peptide binding sites (28-31).

In many kinases, SH3 and SH2 domains are in close proximity to each other. In Src and Tec families, the domains have only a few intervening residues and only have few intramolecular contacts in the Lck SH2-SH3 dimer (101).

Binding specificities of several SH2 domains have been determined by using phoshotyrosine peptide libraries (102, 103). We have predicted the Btk SH2 domain sequence to be pYEXL/I. These peptides were docked into the binding site (71). The sites for phosphotyrosine and residue +1 are formed predominantly by charged residues, whereas the site +3 is mainly hydrophobic. When searching databases, the isoleucine peptide was found in Hck and both YEXI and YEXL motifs of βARK-1 and βARK-2 (71). The modeled Btk SH2 domain with pYEAI peptide is seen in Fig. 5. While both Btk and βARK contain PH domain which can interact with $G_{\beta\gamma}$ it is not known if these proteins interact.

6.5. Kinase domain

The kinase domain of about 250 residues is the only catalytic part in most kinases including the Tec family PTKs. The 3D structure has been solved for several protein kinases. The first 3D structure was for cAPK, which was subsequently refined and crystallized with cofactors and inhibitors (104-108). Although overall sequence similarities are generally low, all the known protein kinases share several conserved residues (109). The known 3D structures have the same scaffolding consisting of two lobes, where ATP is bound in a cleft between the two lobes and substrate interacts mainly with the lower lobe.

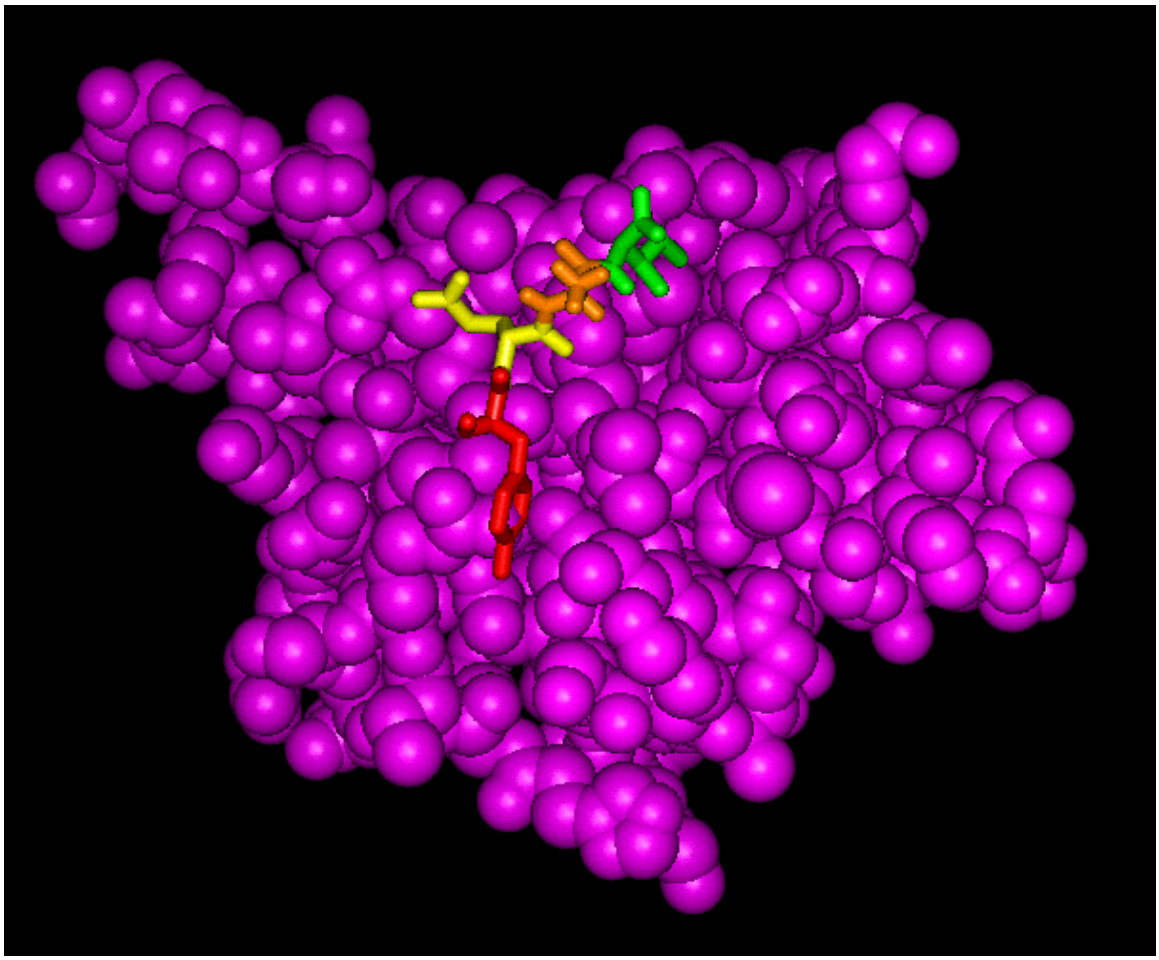

Figure 5. The Btk SH2 domain model with the putative binding peptide, pYEAI (71). The binding residues are indicated with different colors.

Protein kinases are generally regulated by phosphorylation in the activation loop. In cAPK, the phosphothreonine is highly coordinated by residues from the activation and catalytic loops (104, 110, 111). When the enzyme is activated, the upper lobe rotates to lock the ATP molecule between the two domains (112). The ATP binding residues are the most conserved sites in all protein kinases suggesting that both PSKs and PTKs have the same direct in-line reaction mechanism (111, 113).

Btk kinase domain was originally modeled based on the cAPK structure (70) and subsequently based on the IRK and FGF. The models have beenused to study the functional implications of XLA causing mutations (28-31, 70, 114, 115). The kinase domain model was also used to design a novel mutation, that altered the enzyme activity in a predictable fashion (116). Residue, W563, is rather conserved and according to the available models, it is sandwiched between residues R562 and A582 (70). Although W563 and the two surrounding amino acids are not directly involved in catalysis, mutations in the lining residues cause XLA (70), presumably by affecting the orientation of the W563 side chain. Conservative mutation W563F inactivated the enzyme in a predicted manner (116). The expressed protein had no kinase activity, but it presumably folded correctly.

Almost half of the XLA-causing mutations are in the kinase domain which forms more than 40% of Btk. The mutations are almost generally distributed along the Btk sequence, except for the upper lobe, which forms about one third of the domain's length incorporating only 16% of the kinase domain mutations (31). Putative structural description has been given for each XLA mutation (28-30). There are several different types of missense mutations affecting structural, functional and interacting residues. The Btk kinase domain models in Fig. 6 indicate the distribution of the mutations along the polypeptide chain. The severe XLA mutations are mainly in the ATP-binding cleft, the putative substrate binding region or in other functionally or structurally crucial sites (29, 31).

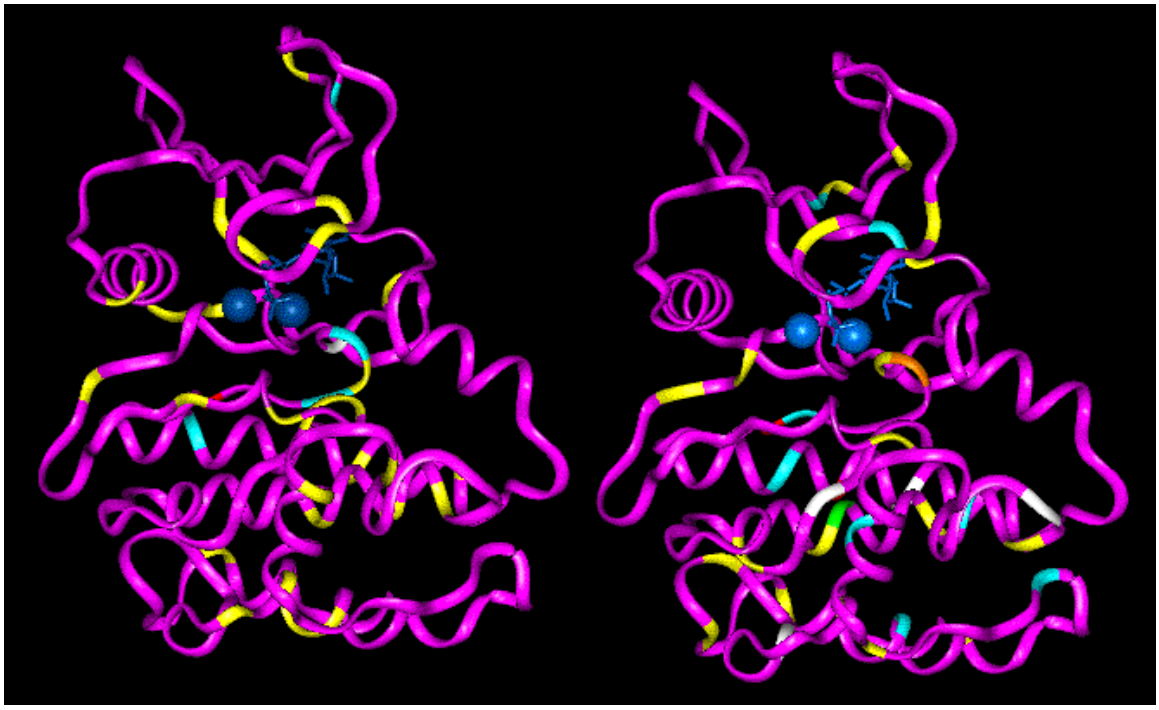

Figure 6. Btk kinase domain model (70). On the left side of the stereo pair the XLA causing missense mutations are shown with color coding for a number of unrelated families. To the right, the other types of mutations are color coded in the kinase domain. The number of affected families is indicated as follows: one family, yellow; two families, cyan; three families, green; four families, white; five families, orange and six or more families in red.

Milder XLA causing mutations can be further away from the functional regions, with some exceptions (29, 31). The very same mutation causes sometimes classical XLA in one patient and only a mild one in another.

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