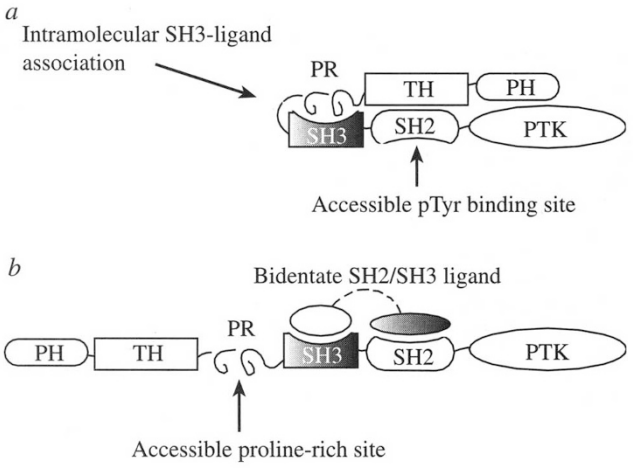

FIG. 5 *a*, Model of the observed intramolecular interaction, showing the observed interaction between the Itk proline-rich region and SH3 domain as well as an interaction between the Itk TH and SH2 domains. *b*, Model of the opening of the intramolecular complex by interaction with a bidentate ligand for the Itk SH3 and SH2 domains.

points directly at acidic residues within the Tec loop (Fig. 1*d*). In addition, the proline-rich region of Itk contains predicted MAP kinase[24], casein kinase II[25] and protein kinase C[26] phosphorylation sites. An analogy for this intramolecular model is found in the Src family kinases, in which phosphorylation of a C-terminal tyrosine residue is thought to regulate kinase activity and substrate binding[9,10] through an intramolecular association with the SH2 domain. Similar intramolecular regulatory interactions have also been identified in the adaptor protein Crk[27]. It is provocative that Tec family members do not contain the regulatory phosphorylation site present in Src family kinases, but may be regulated instead through the described intramolecular interaction between the SH3 domain and proline-rich region. We suggest that this may be a general mechanism by which the activity of Tec family kinases is controlled. □

Methods

Preparation of fusion proteins. Inserts encoding the desired domains of Itk were generated by PCR and were subcloned into the *Bam*HI site of pGEX-2T. The TEC, TH, SH3-KPL, SH3 and SH2 5′ primers encode Itk products beginning at amino acids 97, 109, 154, 171 and 231, respectively. The residue numbering system used throughout the text is that of full-length Tsk/Itk[1]. Point mutations were introduced by a combination of PCR-directed mutagenesis and site-directed mutagenesis. The 3* mutation inactivates the proline-binding pocket of the SH3 domain by converting Trp 208 to Lys. The TH* mutation converts two highly conserved Cys residues in the TH domain (Cys 132 and Cys 133) to Arg residues. The PR* mutation converts Pro 158 and Pro 159 to Ala residues. All fusion proteins were produced in *E. coli* DH5α[16], with the exception of TEC2 and variants thereof. To obtain suitable levels of undegraded protein, the TEC2 fusion proteins were grown in the *E. coli* strain BL21(DE3) in the presence of pUBS1560, a kanamycin-resistant vector that encodes tRNAs for the rare Arg codons AGA and AGG[28].

Collection of NMR spectra and determination of structure. Uniformly labelled samples of $^{15}N/^{13}C$ ItkSH3 and ItkSH3-KPL were expressed using the pGEX-2T expression vector and purified[13–15]. The NMR samples contained ~2.5 mM protein in a D_2O or 90% H_2O/10% D_2O buffer containing 50 mM potassium phosphate, pH 6.0, 150 mM KCl, 0.1 mM DTT, 0.1 mM EDTA and 0.02% NaN_3. Spectra were recorded on a Bruker DMX500 spectrometer at 300 K. Protein resonances were assigned by a series of two- and three-dimensional NMR experiments[13–15]. Distance (NOE) and dihedral (ϕ and χ_1) restraints derived from the NMR experiments were used to calculate structures with the program X-PLOR[29]. To calculate the family of structures, a total of 672 NOE restraints, 22 of which were identified between the KPLPPTP sequence and the Itk SH3 binding pocket, and 55 dihedral restraints were used. Hydrogen-bond distances (based on slowly exchanging amide protons) and a salt bridge restraint (E189–K155, based on previous SH3-ligand structures[13,14]) were included in the final stage of calculation. The average structure was calculated from the coordinates of the 12 final structures and refined with 500 steps of steepest-descent energy minimization with the simulated annealing parameters. The van der Waals energy of the refined average structure is −47.6 kcal m^{-1}, as calculated by CHARMM-19 parameters in X-PLOR[29]. The structures are drawn with the program Ribbons 2.2 (ref. 30).

Preparation of Jurkat lysates and binding assays. Jurkat cells were labelled with ^{35}S-Met and lysed[16]. Tyrosine phosphorylated lysates were prepared from Jurkat cells mock- or OKT3-stimulated for 2 min (ref. 16), or from Jurkat cells stimulated for 10 min at 37 °C with 500 μM pervanadate. All binding assays were performed and analysed as previously described[16].

Immunoblots. All immunoblots were performed as described previously[16]. Antibodies to Sam68 and Grb-2 were from Santa Cruz Biotechnology (sc-333 and sc-255).

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CORRECTION

Identification of the homologous beige and Chediak–Higashi syndrome genes

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THE Genbank accession number of the mouse beige gene (*Lyst*) was incorrect. It is L77884. The Genbank accession number of the human Chediak–Higashi syndrome gene (LYST) is L77889.