p150$^{\text{TS}}$, a Conserved Nuclear Phosphoprotein That Contains Multiple Tetratricopeptide Repeats and Binds Specifically to SH2 Domains*

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Src homology 2 (SH2) domains are structural modules that function in the assembly of multicomponent signaling complexes by binding to specific phosphopeptides. The tetratricopeptide repeat (TPR) is a distinct structural motif that has been suggested to mediate protein-protein interactions. Among SH2-binding phosphoproteins purified from the mouse B cell lymphoma A20, a 150-kDa species was identified and the corresponding complementary DNA (cDNA) was molecularly cloned. This protein encoded by this cDNA, which we have termed p150$^{\text{TS}}$ (for TPR-containing, SH2-binding phosphoprotein), is located predominantly in the nucleus and is highly conserved in evolution. The gene encoding p150$^{\text{TS}}$ (Tsp) was mapped to chromosome 7 of the mouse with gene order: centromere-Tyr-Wnt11-Tsp-Zp2. The amino-terminal two-thirds of p150$^{\text{TS}}$ consist almost entirely of tandemly arranged TPR units, which mediate specific, homotypic protein interactions in transfected cells. The carboxyl-terminal third of p150$^{\text{TS}}$, which is serine- and glutamic acid-rich, is essential for SH2 binding; this interaction is dependent on serine/threonine phosphorylation but independent of tyrosine phosphorylation. The sequence and binding properties of p150$^{\text{TS}}$ suggest that it may mediate interactions between TPR-containing and SH2-containing proteins.

Tyrosine-dependent interactions, phosphoserine/threonine-dependent binding to SH2 domains has also been reported (6–9). In Bcr-Abl chimeras that are implicated in the pathogenesis of chronic myelogenous leukemia, the Bcr segment contains serine/threonine- and glutamic acid-rich regions that bind SH2 domains in a phosphorylation-dependent manner but independent of phosphorytrosine (6). Phosphotyrosine-independent binding of Raf-1 to the SH2 domains of Fyn and Src has also been described (8). More recently, we described SH2 binding by the cyclin-dependent kinase homologue p130$^{\text{PIPSLRE}}$ (9). This interaction is mediated by a serine- and glutamic acid-rich region of p130$^{\text{PIPSLRE}}$ and is likely to involve the same site in the SH2 domain that binds phosphorytrosine-containing peptides.

The tetratricopeptide repeat (TPR) is a 34-amino acid motif found in proteins that function in diverse processes, including cell cycle control, transcriptional repression, protein transport, and protein dephosphorylation (10). TPRs contain eight consensus residues whose size, hydrophobicity, and spacing are conserved. TPRs are predicted to form a pair of amphipathic $\alpha$-helical domains (A and B) that have been proposed to mediate TPR-TPR interactions (11, 12). While there is as yet no evidence that TPR motifs interact directly, they have been shown to participate in interactions between TPR-containing proteins. For example, the TPR-containing proteins CDC23 and CDC27 form part of a complex that promotes anaphase (13, 14); a mutation in the TPR region of CDC27 impairs its ability to interact with CDC23 (14). There is also evidence that TPRs mediate interactions with non-TPR-containing proteins: the transcriptional repression protein SSN6 (Cyc8), for example, interacts with specific DNA-binding proteins by means of its TPR region (15).

In work described here, SH2-binding phosphoproteins from the B-lymphoid cell line A20 were isolated by affinity chromatography. Internal peptide sequences from one of these proteins were used to molecularly clone a complementary DNA that encodes a hitherto unidentified protein of 150 kDa. This protein, which we have termed p150$^{\text{TS}}$ (for TPR-containing, SH2-binding phosphoprotein), contains 1173 amino acid residues and is located predominantly in the nucleus. The aminoterminal portion of p150$^{\text{TS}}$ contains a tandem array of 15 TPRs; the TPR-containing region mediates p150$^{\text{TS}}$ self-association in transfected cells. Specific binding of p150$^{\text{TS}}$ to SH2 domains is mediated by a serine- and glutamic acid-rich region near the carboxyl terminus. This interaction requires serine/threonine phosphorylation but is independent of tyrosine phosphorylation. The sequence and binding properties of p150$^{\text{TS}}$ suggest that it may mediate interactions between TPR-containing and SH2-containing proteins.
MATERIALS AND METHODS
Protein Isolation and Peptide Sequencing—SH2-binding proteins
were isolated from the B-lymphoid cell line A20 by affinity chromatogra-
phy, fractionated by electrophoresis, and transferred to PVDF mem-
branes as described previously (9). Generation, isolation, and sequenc-
ing of tryptic peptides were performed by Drs. David F. Reim and David
W. Speicher, Wistar Protein Microsequencing Facility, Philadelphia,
PA. Interrogation of protein sequenced databases was carried out at
the National Center for Biotechnology Information (NCBI) using the
BLAST network service.

Fig. 1. Sequence of p150TSP cDNA. A, the nucleotide sequence of p150TSP cDNA, derived from the overlapping clones 19-4, 17-3, and 13-1, is
shown on the upper line, the conceptual translation of the p150TSP open reading frame is shown on the lower line. Matches to peptide sequences
derived from affinity-purified p150TSP are underlined. B, organization of TPRs in p150TSP. TPR consensus residues are indicated in
bold type. Individual TPR motifs are numbered at the left. The canonical TPR consensus sequence as defined in Ref. 10 is shown at the
bottom.
Isolation of Complementary DNA Clones Encoding p150TSP—Based on the sequence of peptide 1 (VQADVPPEILNNVGALHFR), a unique, 57-mer oligonucleotide probe (5\textsuperscript{9} GTG CAG GCT GAT GTG CCC CCT GAGATCCTGAACAATGTGGGCGCCCTGCACTTCCGG3\textsuperscript{9} 3\textsuperscript{108} cpm/mg) was synthesized. The probe was labeled with \(^{32}\text{P}\) using T4 polynucleotide kinase to a specific activity of \(5 \times 10^9\) cpm/mg and hybridized to \(5 \times 10^5\) recombinant bacteriophage from a mouse spleen cDNA library in the vector LambdaZapII (Stratagene). Hybridization was carried out overnight at 45°C in 6x SSC, 20% formamide, 5x Denhardt’s solution, 10% dextran sulfate (Pharmacia Biotech Inc.), 0.1% SDS and 100 \(\mu\)g/ml salmon sperm DNA at an oligonucleotide concentration of 1 \(\mu\)g/ml.

Filters were washed twice for 6 min in 2x SSC, 0.1% SDS at room temperature and twice for 6 min in 2x SSC, 0.1% SDS at 45°C. Positive bacteriophage were isolated by two additional rounds of plaque purification, and cDNAs were recovered as recombinant pBluescript plasmids, using an excision protocol supplied by the manufacturer.

Recombinant plasmid DNA was carried through a second round of hybridization screening. Based on the sequences of peptide 2 (QXS-DLLSQAQYHVA) and peptide 3 (DKGNFYEASDVFK), degenerate oligonucleotide probes SD945 (5\textsuperscript{9} CA(A/G)GC(A/C/T/G)CA(A/G)TA(C/T) CA(C/T) GT 3\textsuperscript{9}) and SD944 (5\textsuperscript{9} GA(T/C) AA(A/G) GG(A/T/G/C) AA(T/C) TT(T/C) TA(T/C) GA 3\textsuperscript{9}) were synthesized.
peptides 2 and 3, respectively, were synthesized. These were labeled with $^{32}\text{P}$ and hybridized sequentially to plasmid DNA that had been digested with Sall and NotI, fractionated by agarose gel electrophoresis, and transferred to nitrocellulose. Hybridization was carried out overnight in 6 x SSC, 0.1% SDS, and 100 g/ml salmon sperm DNA at an oligonucleotide concentration of 33 $\mu$g/liter; hybridization was performed at 42°C for SD945 and at 44°C for SD944. Membranes were washed in 2 x SSC, 0.1% SDS twice for 6 min at room temperature and then once for 8 min at 42°C (for SD945) or once for 10 min at 44°C (for SD944). Between hybridizations, the membranes were wiped of probe by heating for 30 min at 68°C in 0.1 x SSC, 0.1% SDS.

Mapping of the Mouse Tsp Gene—For genetic mapping we analyzed the progeny of the cross (NFS/N x Mus spretus) x M. spretus or C57Bl/6J, which have been typed for more than 650 markers, including the chromosome 7 markers Tyr (tyrosinase), Wnt11 (wingless-related gene 11), and Zp2 (zona pellucida 2) (16, 17). Parental mouse DNAs were screened for restriction fragment length polymorphisms of Tsp using a full-length Tsp cDNA probe (clone 19-4). The Tsp probe detected HindIII fragments of 8.6, 6.3, 3.0, 2.5, and 2.4 kb in NFS/N and C57Bl/6J, and HindIII fragments of 9.2, 7.0, 2.9, 2.5, and 2.4 kb in Mus spretus.

For immunofluorescence, affinity-purified Ab1543 and Ab1544 or total IgG from the corresponding preimmune sera were used at 0.3 $\mu$g/ml in KB + Nonidet P-40. Mouse anti-p150TSP antibody Ab635 and mouse preimmune serum were used at 1:500 dilutions in KB + Nonidet P-40. For double immunofluorescence experiments, microtubules were stained with the mouse anti-tubulin antibody TU27B (19) at 1:75 dilution or with rabbit anti-tubulin antibody Ra53 (provided by Dr. D. Murphy, Johns Hopkins University School of Medicine) at 1:50 dilution. Secondary reagents used in these experiments included fluorescein isothiocyanate-conjugated horse anti-mouse IgG (Vector Laboratories, Burlingtom, CA) and fluorescein isothiocyanate-conjugated swine anti-rabbit IgG or biotinylated horse anti-mouse IgG (Vector Laboratories) which were used, in addition with Texas red-streptavidin (Life Technologies, Inc.). For competition experiments, 150 $\mu$l of diluted, primary antibodies were preincubated for 30 min with 15 $\mu$g of a purified GST fusion protein containing residues 1059–1173 of p150TSP.

Generation of Epitope-tagged p150TSP—The nonapeptide influenza hemagglutinin (HA) epitope YPYDVPDYA, which is recognized by the mouse monoclonal antibody 12CA5, was fused to the carboxyl terminus of p150TSP as follows. The 4.2-kb Sall-NotI insert from clone 19-4 was cloned into the vector pET-21 (Novagen) to yield pET-21-p150TSP. Oligonucleotides 5' CAT GTG CAC GGC CGG GTG 3' (sense) and 5' TAT TTT TTT CCG GCG GCT CAT ACG GTC GAC GTG ATG TGG TGA GTC GCT ATC ATG CCC ATG 3' (antisense) were used as polymerase chain reaction primers to amplify a 1.1-kb fragment from pET-21-p150TSP. The resulting cassette was exchanged for the SrfI-NotI fragment of pET21-p150TSP. The entire Sall-NotI insert, encoding p150TSP fused at its carboxyl terminus to the HA epitope, was then subcloned into pCIS2 to yield pCIS2-p150TSP-HAC.

Expression of p150TSP and GST-SH2 Binding Assays—The wild-type Tsp coding sequence was introduced into the expression vector pCIS2 to yield pCIS2-p150TSP. A series of deletion mutations were constructed by polymerase chain reaction, and the resulting mutant cDNAs were introduced into pCIS2. Plasmids encoding amino-terminal truncations, the codons deleted were replaced with the sequence 5’ ATG GGG 3’, which encodes the dipeptide Met-Gly. Mutations included deletion of codons 1–886 (p150(887–1173)), 1–496 (p150(497–1173)), and 822–1173 (p150(1–821)). For immunoblotting, Ab1544 and Ab1543 were each used at 1 $\mu$g/ml in KB buffer.

Secondary reagents used in these experiments included fluorescein isothiocyanate-conjugated horse anti-mouse IgG (Vector Laboratories, Burlingtom, CA) and fluorescein isothiocyanate-conjugated swine anti-rabbit IgG or biotinylated horse anti-mouse IgG (Vector Laboratories) which were used, in addition with Texas red-streptavidin (Life Technologies, Inc.). For competition experiments, 150 $\mu$l of diluted, primary antibodies were preincubated for 30 min with 15 $\mu$g of a purified GST fusion protein containing residues 1059–1173 of p150TSP.

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For immunoblotting, Ab1543 and Ab1544 or total IgG from the corresponding preimmune sera were used at 0.3 $\mu$g/ml in KB + Nonidet P-40. Mouse anti-p150TSP antibody Ab635 and mouse preimmune serum were used at 1:500 dilutions in KB + Nonidet P-40. For double immunofluorescence experiments, microtubules were stained with the mouse anti-tubulin antibody TU278 (19) at 1:75 dilution or with rabbit anti-tubulin antibody Ra53 (provided by Dr. D. Murphy, Johns Hopkins University School of Medicine) at 1:50 dilution. Secondary reagents used in these experiments included fluorescein isothiocyanate-conjugated horse anti-mouse IgG (Vector Laboratories, Burlingtom, CA) and fluorescein isothiocyanate-conjugated swine anti-rabbit IgG or biotinylated horse anti-mouse IgG (Vector Laboratories) which were used, in addition with Texas red-streptavidin (Life Technologies, Inc.). For competition experiments, 150 $\mu$l of diluted, primary antibodies were preincubated for 30 min with 15 $\mu$g of a purified GST fusion protein containing residues 1059–1173 of p150TSP.

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A Conserved TPR-containing Protein That Binds SH2 Domains

To assay SH2 binding by endogenously expressed p150TSP, A20 cells (2 × 10⁶) were washed twice in phosphate-buffered saline and lysed in D buffer (100 mM NaCl, 25 mM Tris-Cl (pH 8.0), 1 mM EDTA, 1 mM Na₂VO₄, 1 mM Na₂MoO₄, 1 mM phenylmethylsulfonyl fluoride, 1% Nonidet P-40, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 5 μg/ml pepstatin). GST-BlkSH2 binding reactions were carried out as above. In some experiments, the peptide SD12 (TWPAKSEQQVRXKGRSTPPRDP-EGGLG) was used as a nonspecific competitor.

Phosphorylation Dependence of SH2 Binding by p150TSP—p150TSP was expressed in 293 cells and labeled metabolically with [³²P]P. Cells (2 × 10⁶) were lysed in 10 ml of C buffer (100 mM NaCl, 25 mM Tris-Cl (pH 7.6), 25 mM NaF, 1 mM EDTA, 2 mM Na₂VO₄, 1 mM Na₂MoO₄, 1 mM phenylmethylsulfonyl fluoride, 1% Nonidet P-40, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 5 μg/ml pepstatin), and lysates were clarified by centrifugation. p150TSP was immunoprecipitated in reactions containing 1 ml (2 × 10⁶ cell eq) of lysate and 10 μg each of Ab1543 and Ab1544, affinity-purified against a GST-ASepharose beads. Immunoprecipitations were carried out for 3 h at 4°C; beads were washed by centrifugation and washed four times for 6 min each with 1 ml of C buffer. Immunoprecipitates were treated with calf intestine alkaline phosphatase (Sigma) as described (9). Samples were split into three aliquots, fractionated by electrophoresis, and transferred to PVDF membranes. One membrane was immunoblotted with a mixture of antibodies Ab1604 and Ab1544, and the remaining membranes were assayed for binding to biotinylated GST or GST-BlkSH2 fusion protein (9).

RESULTS

Isolation of p150TSP by SH2 Affinity Chromatography and Molecular Cloning of TSP cDNA—We have shown previously that SH2 domains from the tyrosine kinases Blk, Fyn(T), and Lyn bind distinct sets of phosphoproteins from the B-lymphoid cell line A20 (21) and have described the use of preparative scale SH2 affinity chromatography to identify specific SH2 ligands by protein microsequence analysis and molecular cloning (9). Among the SH2 ligands identified in lysates from A20 cells, we detected a phosphoprotein of apparent molecular mass 150 kDa. Amino acid sequences of six internal tryptic peptides from this protein were obtained (Fig. 1A). Based on one of these peptides, P1 (VQADVPPEILNNVGALHFR), we designed a 57-mer oligonucleotide probe of unique sequence. This was used to screen 5 × 10⁶ recombinant bacteriophage from a mouse spleen cDNA library. Twenty positive clones were isolated and used to establish a second round of screening by hybridization to two degenerate oligonucleotides, corresponding to residues 8 through 13 of peptide P2 (QAQHYV) and residues 1 through 7 of peptide P3 (DKGNFYE). Four clones hybridized to both degenerate oligonucleotides, including clones 19-3 (3591 base pairs) and 14-4 (4167 base pairs). The sequences of these and two overlapping clones, 13-1 and 17-3, define an open reading frame of 1173 codons, specifying a protein that we have termed p150TSP (Fig. 1A). The p150TSP protein sequence can be divided into two regions: the amino-terminal 815 residues contain 15 occurrences of the 34-amino acid TPR motif; the carboxyl-terminal 358 residues are rich in serine, glutamic acid, and aspartic acid and contain multiple potential casein kinase 2 phosphorylation sites (Fig. 1B). While the Tsp coding sequence predicts a protein with a molecular mass of 129 kDa, transcription and translation of Tsp cDNA in vitro yielded a predominant product whose apparent molecular mass was 150 kDa, in agreement with the size of the protein originally purified from A20 cells (data not shown) and with the size of endogenously expressed p150TSP as detected by immunoblotting (see below).

Interrogation of nucleotide and protein sequence data bases using the TBLASTN algorithm (22) revealed 98.6% amino acid sequence identity between p150TSP and a hypothetical, 1173-codon open reading frame in the human genome (GenBank™ accession number D63875). In addition, p150TSP was found to share 31% amino acid sequence identity with a hypothetical, 1245-amino acid protein encoded at locus B0464.2 of Caenorhabditis elegans (23). The homology between p150TSP and the putative B0464.2 product extends from near the amino terminus (residue 14 of p150TSP) through the TPR-rich region and includes most of the carboxyl-terminal domain (to residue 1111 of p150TSP) (Fig. 2). Thus, B0464.2 is likely to encode a C. elegans homologue of p150TSP. Remarkably, interrogation of the dbEST data base of expressed sequence tags (24) revealed homology between p150TSP and the conceptual translation of an expressed sequence tag from the higher plant Arabidopsis thaliana (T46289; 47% identity over 204 residues). The similarity between p150TSP and the arabidopsis expressed sequence tag includes a TPR unit but extends beyond it (Fig. 2). This suggests that a progenitor of p150TSP first appeared before the animal and plant kingdoms diverged. Consistent with this suggestion, a TBLASTN search also detected a hypothetical, 1045-amino acid open reading frame in Saccharomyces cerevisiae (AOE1045) that exhibits significant (smallest sum probability P(N) = 1.4 × 10⁻⁵⁵ N = 12) homology with p150TSP (Fig. 2). The existence of p150TSP homologues in nematodes, plants, and yeast indicates an extraordinary degree of evolutionary conservation.

Mapping of the Tsp Gene to Mouse Chromosome 7—To map the Tsp gene in the mouse, DNA samples from the progeny of a multilocus cross were examined for inheritance of a restriction enzyme length variant of Tsp as described under “Materials and Methods.” The observed pattern of inheritance was compared with that of 650 markers, including the chromosomes 7 markers Tyr (tyrosinase), Wnt11, and Zp2 (zona pellucida glycoprotein 2). Tsp was mapped to a position on chromosome 7 proximal to Zp2. The data indicate the following gene order and distances: Tyr-4.7 ± 2.0-Wnt11-6.0 ± 2.4-Tsp-3.1 ± 1.8-Zp2.

Expression of Tsp RNA in Mouse Tissues—A probe specific for Tsp RNA was radiolabeled and hybridized to polyadenylated RNA from various mouse tissues. The Tsp probe detected a single RNA species of about 5.0 kb in every tissue examined (Fig. 3). This transcript is somewhat longer than the longest Tsp cDNA clone obtained (4.2 kb) suggesting that 5' or 3'- untranslated sequences are incompletely represented in the cDNA. This interpretation is consistent with the observation that the cDNA sequence lacks a polyadenosine tract.

Expression of p150TSP and Binding to an SH2 Domain—To obtain additional evidence that the Tsp open reading frame encodes a physiologic gene product, we used antibodies directed against proteins encoded by Tsp to detect immunoreactive species in cell lysates. Rabbit antibody Ab1544, which was raised against a GST fusion containing residues 1059–1173 of p150TSP, detected a 150-kDa protein in total lysates of the B-lymphoid cell lines A20 and WEHI231 (Fig. 4, lanes 3 and 4). This antibody also detected a comigrating species among proteins from A20 cells that were specifically retained by a GST-BlkSH2 affinity matrix (Fig. 4, lane 6); this species was not detected in eluates from an affinity matrix containing GST alone (Fig. 4, lane 5). Expression of Tsp cDNA by transfection into 293 cells yielded a 150-kDa protein that was immunoreactive with Ab1544 and which comigrated with the endogenous, 150-kDa species from A20 and WEHI231 cells (Fig. 4, lane 1); longer exposure revealed a similar immunoreactive species in the extract of 293 cells transfected with vector alone (data not shown), which likely represents endogenous p150TSP. In the A20 cell lysate, and to a lesser extent in the WEHI231 lysate, an additional species of about 120 kDa was also observed (Fig. 4, lanes 3 and 4); this may represent a proteolytic product of p150TSP, as its yield was variable. The discrepancy between the predicted and observed mobilities of p150TSP may reflect anomalous electrophoretic mobility caused by the acidic region. Phospho-
rylation also contributes to this difference, as dephosphorylation of p150TSP in vitro results in a 5-kDa diminution in apparent molecular mass (see Fig. 8).

p150TSP Is Localized to the Cell Nucleus—The intracellular distribution of endogenous p150TSP in NIH3T3 cells was examined by immunofluorescence microscopy (Fig. 5). In interphase cells stained with the rabbit anti-p150TSP antibody Ab1544, speckled nuclear fluorescence was observed (Fig. 5A); fluorescence was reduced to background by an excess of the corresponding specific antigen (Fig. 5B). Similar nuclear staining was seen in cells probed with a mouse anti-p150TSP antibody directed against residues 1–821 of p150TSP, but not with the corresponding preimmune serum (Fig. 5D). We conclude that p150TSP accumulates predominantly or exclusively in the cell nucleus.

Specificity of SH2 Binding by p150TSP—The experiment of Fig. 4 demonstrated that endogenously expressed p150TSP is retained by an SH2 affinity matrix; we proceeded to examine the specificity of this interaction. p150TSP was expressed by transfection in 293 cells and labeled metabolically with 32P. Specificity of SH2 binding by p150TSP is demonstrated by the ability of the antibody to probe for the presence of p150TSP in association with SH2 domains in transfected cells.

### Fig. 2. Comparison of mouse p150<sup>SP</sup> and putative p150<sup>SP</sup> homologues.

Conserved TPR-containing proteins that bind SH2 domains.
A Conserved TPR-containing Protein That Binds SH2 Domains

To define the region of p150TSP responsible for SH2 binding, we tested a series of p150TSP deletion mutants for retention by an SH2 affinity matrix. Proteins were expressed by transfection in 293 cells and labeled metabolically with [35S]methionine and [35S]cysteine. Expression and intracellular accumulation of each p150TSP fragment was verified (data not shown). Cell lysates were adsorbed to a GST-BlkSH2 affinity matrix; retained proteins were fractionated by SDS-PAGE and visualized by autoradiography. Wild-type p150TSP (Fig. 7, lane 2) and fragments of p150TSP spanning residues 497-1173 (Fig. 7, lane 5) or residues 887-1173 (Fig. 7, lane 6) were retained by the SH2 affinity matrix; fragments spanning residues 1-821 (Fig. 7, lane 3) or residues 497-821 (Fig. 7, lane 4) were not retained. In this way, the SH2 binding site(s) of p150TSP was localized to the interval between residues 887 and 1173. Because this region is devoid of tyrosine residues, it seemed likely that the binding of p150TSP to SH2 is independent of phosphotyrosine; it remained formally possible, however, that binding was not direct but rather mediated by a third protein. To determine whether p150TSP bound the SH2 domain directly, and whether this interaction was dependent on phosphorylation of p150TSP, we used a filter immobilization assay.

SH2 Binding by p150TSP Is Direct and Dependent on Phosphorylation—Wild-type p150TSP was expressed by transfection in 293 cells and labeled metabolically with [32P]. Cells were lysed, and p150TSP was immunoprecipitated with Ab1543 and Ab1544. Immunoprecipitates were treated with calf intestinal alkaline phosphatase (Fig. 8, lanes 1, 3, 5, 7, 9, and 11) or left untreated (Fig. 8, lanes 2, 4, 5, 8, 10, and 12). Then each sample was split three ways and fractionated by SDS-PAGE. Protein was transferred to PVDF membranes and assayed in parallel for binding to biotinylated GST-BlkSH2 fusion protein (Fig. 8, lanes 1-4), biotinylated GST (Fig. 8, lanes 5-8), or an anti-p150TSP antibody mixture (Fig. 8, lanes 9-12). The GST-BlkSH2 protein was observed to bind directly to p150TSP (Fig. 8, lane 2). Treatment of the immunoprecipitates with alkaline phosphatase, however, substantially reduced binding of the GST-BlkSH2 fusion protein to p150TSP (Fig. 8, lane 1). Phosphatase treatment reduced the amount of p150TSP-associated phosphate by about 5-fold (Fig. 8, compare lanes 4, 8, and 12 to lanes 3, 7, and 11) but did not significantly affect the recovery of p150TSP (Fig. 8, compare lanes 9 and 10). Binding of the GST-BlkSH2 protein to p150TSP was dependent on the SH2 moiety, as little or no binding was observed with biotinylated GST alone (Fig. 8, lanes 5 and 6). Thus, the binding of BlkSH2 to p150TSP is direct and phosphorylation-dependent. Because p150TSP truncation mutants lacking tyrosine retain their ability to bind SH2 (Fig. 7), we conclude that SH2 binding by p150TSP requires phosphorylation at serine or threonine residues.

Self-association of p150TSP in Transfected Cells—Based on secondary structure predictions, TPR motifs have been proposed to mediate homotypic interactions (11, 12) and have been shown to participate in the formation of complexes between TPR-containing proteins (14). The presence of an extensive TPR-containing region suggested that p150TSP might undergo self-association. To test this, p150TSP was tagged at its carboxyl terminus with a 9-amino acid influenza HA epitope (p150TSP-HAC) and coexpressed in 293 cells with fragments of p150TSP spanning residues 1-821, 497-1173, 497-821, or 887-1173. Protein was labeled metabolically with [35S] and immunoprecipitated with the anti-HA antibody 12CA5 in the presence (Fig. 9, lanes 2, 4, 6, and 8) or absence (Fig. 9, lanes 1, 3, 5, and 7) of a specific HA competitor peptide. Immunoprecipitations were carried out in parallel with anti-p150TSP antibody Ab1544 (Fig. 9, lanes 9-12). Precipitated proteins were fractionated by SDS-PAGE and detected by autoradiography. A [35S]-labeled protein corresponding to p150TSP-HAC was precipitated from each of
thetransfectedcelllysatessbythe12CA5antibody(Fig.9, lanes 1, 3, 5, and 7, closed arrow); precipitation of this protein was greatly reduced in the presence of an HA peptide competitor (Fig. 9, lanes 2, 4, 6, and 8). p150TSP fragments comprising residues 1–821, 497–1173, and 497–821 were observed to coprecipitate with p150TSP-HAC (Fig. 9, lanes 1, 3, and 5). The fragment spanning residues 887-1173, however, was not precipitated (Fig. 9, lane 7), despite the fact that all four fragments could be immunoprecipitated from lysates of transfected cells by Ab1544 (Fig. 9, lanes 9–12). Precipitation of fragments 1–821, 497–1173, and 497–821, like that of p150TSP-HAC, was greatly reduced in the presence of the HA peptide (Fig. 9, lanes 2, 4, and 6). Fragments 1–821 and 497–821 were also present in immunoprecipitates of p150TSP-HAC performed with...
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Ab1544 (Fig. 9, lanes 9 and 10). Because Ab1544 recognizes a carboxyl-terminal p150<sup>TPS</sup> epitope, the presence of fragments 497-1173 and 887-1173 in Fig. 9, lanes 11 and 12, is uninformative. Thus, fragments of p150<sup>TPS</sup> derived from the TPR-containing region are able to associate, directly or indirectly, with p150<sup>TPS</sup> in transfected cells.

**DISCUSSION**

We have used SH2 affinity chromatography to isolate SH2-binding proteins from the B-lymphoid cell line A20. By partial peptide sequence determination and molecular cloning, one of these SH2 ligands was identified as a hitherto undescribed, ubiquitously expressed protein of 1173 amino acid residues, which we have termed p150<sup>TPS</sup>. p150<sup>TPS</sup> has a predicted molecular mass of 129 kDa but migrates as a protein of 150 kDa in SDS-polyacrylamide gels; anomalous mobility may be conferred by the acidic, carboxyl-terminal portion of the protein. Residues 74 through 815 of p150<sup>TPS</sup> comprise a tandem array of 15 TPRs, two of these repeats (TPRs 1 and 2; residues 119-154 and 179-216) appear to contain the amino-terminal helical domain (domain A) but not the carboxyl-terminal domain (domain B). The TPR repeat region is interrupted in four places by non-TPR-containing inserts (residues 75-162, between TPRs 1 and 2; residues 445-496, between TPRs 9 and 10; residues 599-646, between TPRs 12 and 13; and residues 715-781, between TPRs 14 and 15).

Comparison of the individual TPR motifs of p150<sup>TPS</sup> provides the consensus (I/L/V)<sub>x</sub>(X/Y)<sub>x</sub>(A/G)<sub>x</sub>(Y/F)<sub>x</sub>(X/E)<sub>x</sub>(X/A)<sub>x</sub>(F/Y)<sub>x</sub>AxxAL/R/K)<sub>x</sub>xXXX. This is in close agreement with the canonical TPR motif, xxxWxxLXXxxYxxxxXXXAxxFxXXAxxxPXxx (11, 12). The p150<sup>TPS</sup> TPR consensus differs from the canonical sequence in that tryptophan is not well conserved at position 4; nonetheless, in 10 out of the 15 TPR motifs in p150<sup>TPS</sup>, hydrophobic residues are found at that position. Another difference from the canonical TPR motif is the poor conservation of proline at position 32. This difference, however, is not unique to p150<sup>TPS</sup>; for example, in the human serine/threonine phosphatase PPP5, only one of four TPRs contains proline at that position (26). Structural, genetic, and biochemical observations have suggested that TPRs mediate formation of specific protein complexes (10). Consistent with these data, we have shown that p150<sup>TPS</sup> undergoes self-association and that this interaction is mediated by the amino-terminal, TPR-containing region. Whether this association is mediated by direct interactions between TPR motifs has yet to be demonstrated.

The carboxyl-terminal acidic region of p150<sup>TPS</sup> mediates binding to SH2 domains. While binding of GST-BikSH2 to filter-immobilized p150<sup>TPS</sup> was observed at a fusion protein concentration of 100 nM, estimation of the affinity of SH2 binding by p150<sup>TPS</sup> is complicated by several factors, including the possible existence of multiple binding sites in the acidic region of p150<sup>TPS</sup>, multimerization of p150<sup>TPS</sup> through interactions between TPR-containing regions, and the ability of GST-SH2 fusion proteins to dimerize. Two lines of evidence indicate that SH2 binding by p150<sup>TPS</sup> is dependent on phosphorylation but independent of phosphotyrosine. First, a 287-amino acid fragment of p150<sup>TPS</sup> which lacks tyrosine residues retains the ability to bind SH2. Second, SH2 binding was greatly reduced when p150<sup>TPS</sup> was dephosphorylated by treatment with an alkaline phosphatase. Despite the lack of a requirement for phosphotyrosine, p150<sup>TPS</sup> appears to interact with the same site on the SH2 domain that binds phospho-
rosine-containing peptides. Binding was abolished by excess free phosphotyrosine and by the phosphotyrosine analogue phenylphosphate (21); furthermore, a phosphotyrosine-containing peptide that binds Src-type SH2 domains with high affinity was able to compete specifically with p150TSP for SH2 binding. Consistent with the results of specific competition experiments, binding of p150TSP was greatly reduced by mutation of a single residue in the Blk FLV/ARES motif, Ser-147, which is predicted on the basis of structural data to interact with phosphotyrosine (27, 28). While it is possible that impairment of p150TSP binding by free phosphotyrosine or the phosphotyrosine-containing peptide reflects an allosteric interaction between separate binding sites, the observation that the Ser-147 mutation also impairs binding makes this interpretation less likely.

We recently showed that another protein, p130PITSLRE also binds SH2 domains in a phosphorylation-dependent, phosphotyrosine-independent fashion (9). SH2 binding by both p150TSP and p130PITSLRE is mediated by an acidic region that contains multiple casein kinase II phosphorylation sites; in the case of p130PITSLRE phosphorylation of bacterially expressed protein by casein kinase II was sufficient to confer SH2 binding ability. While the structural basis of SH2 binding by p150TSP and p130PITSLRE remains to be determined, we note that several potential casein kinase II sites in the acidic regions of these proteins exhibit the amino acid sequence SEEE. Three-dimensional structures of Src and Lck SH2 domains in complex with the high-affinity peptide EPQ(pY)EEIOIYL have been determined (25, 29). In these complexes, the SH2 domain makes critical contacts with glutamic acid residues at Tyr(P) at positions 1 and 2. It is plausible that the SEEE sites in p150TSP and p130PITSLRE when phosphorylated, mimic the high-affinity SH2-binding site (pY)EE1.

The biological significance of phosphotyrosine-independent SH2 interactions has yet to be established, and physiologic ligands of p150TSP and p130PITSLRE have not yet been identified. We have been unable to co-immunoprecipitate Blk and p150TSP, and p150TSP does not appear to be a substrate for slgG-activated tyrosine kinases. Nonetheless, the ability of p150TSP and p130PITSLRE to bind SH2 domains in a phosphorylation-dependent, phosphotyrosine-independent fashion suggests that the number of proteins that interact with the classical phosphopeptide binding sites of SH2 domains may be substantially larger than appreciated.

Proteins homologous to p150TSP can be found in other species. A putative C. elegans coding sequence specifying a protein 31% identical with p150TSP. In its overall structure, including the arrangement of the TPR motifs and the sequence of the acidic region, the hypothetical C. elegans homologue resembles p150TSP. In general, the homology between the nematode and mouse TPR motifs extends beyond the consensus residues; an exception is the seventh repeat, which is apparently not conserved in the nematode protein. The gene that encodes p150TSP in the mouse was mapped to chromosome 7 between Wnt11 and Zp2; the putative C. elegans coding sequence is located on chromosome 3 at locus B0464.2 (23). No mutations in the mouse or in C. elegans have yet been mapped to those loci. A search of the dbEST data base identified a partial cDNA from the flowering plant A. thaliana which, when translated, specifies a 68-amino acid sequence with 47% identity to p150TSP. Strikingly, an anonymous, 1045-amino acid open reading frame in the genome of S. cerevisiae (30) also exhibits significant homology to p150TSP. The TPR-containing region of the hypothetical yeast protein is most similar to that of mouse p150TSP in regions corresponding to the second, tenth, thirteenth, and fourteenth repeats of the mouse protein. Homology between the yeast and mouse proteins is not restricted to TPR consensus residues or to the TPR-containing region, suggesting that the yeast protein is a homologue of mouse p150TSP and indicating an extraordinary degree of evolutionary conservation. While the function of p150TSP in higher eukaryotes is unknown, we have found that homologous disruption of the yeast homologue is associated with mitotic chromosomal instability and temperature-sensitive defect in cell growth.

In recent years, it has become apparent that assembly of a diverse group of multicomponent protein complexes is mediated by a relatively small number of conserved structural modules, such as SH2 and SH3 domains, that bind specific target sites with high specificity (31). Some proteins contain multiple ligand-binding modules and apparently function as linking molecules. GRB-2, for example, which contains two SH3 domains and a single SH2 domain, functions as a bridge between transmembrane signaling complexes and SOS, a guanine nucleotide exchange factor for p21ras (32–36). The presence of TPR motifs and an SH2-binding region within p150TSP suggests that this protein may be able to mediate interactions between TPR-containing and SH2-containing proteins.

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