Journal of Cellular Biochemistry 59:418–430 (1995)

Molecular Cloning of a Mouse Epithelial Protein-Tyrosine Phosphatase With Similarities to Submembranous Proteins

Wiljan Hendriks, Jan Schepens, Dietmar Bächner, Johan Rijss, Patrick Zeeuwen, Ulrich Zechner, Horst Hameister, and Bé Wieringa

Department of Cell Biology and Histology, University of Nijmegen, 6500 HB Nijmegen, The Netherlands (W.H., J.S., J.R., P.Z., B.W.); Department of Clinical Genetics, University of Ulm, D-89069 Ulm, Germany (D.B., U.Z., H.H.)

Abstract Protein-tyrosine phosphatases (PTPases) form an important class of cell regulatory proteins. We have isolated overlapping cDNA clones that together comprise an 8 kb transcript encoding a novel murine PTPase which is expressed in various organs. Sequence analysis revealed an open reading frame of 2,460 amino acid residues. The predicted protein, PTP-BL, is a large non-transmembrane PTPase that exhibits 80% homology with PTP-BAS, a recently described human PTPase. PTP-BL shares some intriguing sequence homologies with submembranous proteins. It contains a band 4.1-like motif also present in the tumor suppressors neurofibromatosis 2 and *expanded*, five 80 amino acid repeats also present in the *discs-large* tumor suppressor, and a single catalytic phosphatase domain. No obvious homologies to other proteins were found for the N-terminal region of the protein other than human PTP-BAS. RNA in situ hybridization experiments show that the PTP-BL gene is expressed in epithelial cells, predominantly in kidney, lung, and skin. These data suggest a cell cortical localization for PTP-BL in epithelial cells and a possible role in the morphology and motility of epithelial tissues.

Key words: gene expression, mouse embryo, signal transduction, band 4.1, discs-large homologous region, membrane skeleton, cytoskeleton

Cells of multicellular organisms have the ability to respond to external stimuli, and such responses can lead to apoptosis, quiescence, differentiation, mitosis, or cell movement. Transduction of signals triggered by hormones or growth factors, or by cell-cell or cell-matrix contacts, is commonly mediated by transmembrane molecules. Failure in these processes can lead to improper cell growth or differentiation and tumor formation. Indeed, defects in components of growth factor signalling pathways have been demonstrated for many cancer types, and the importance of cell contact-signalling molecules in the process of tumor metastasis is well established [van Roy and Mareel, 1992]. In addition, evidence is accumulating that defects in cell-cell and cell-matrix contact signalling can be involved in tumor initiation [Hedrick et al., 1993]. That cell adhesion molecules as well as the corresponding peripheral membrane proteins have a regulatory role in growth control is also demonstrated by studies on tumor suppressor genes in Drosophila [Bryant et al., 1993]. The *discs large* (*dlg*) gene, for example, is needed to enable Drosophila imaginal disc cells to leave the cell cycle and differentiate into polarized cells [Woods and Bryant, 1991]. Absence of the protein in *dlg* mutants therefore leads to uncontrolled proliferation. The DlgA protein is localized in the undercoat of the septate junctions, which are the counterpart of the vertebrate tight junctions. Other cell cortical proteins that exert growth control include the Drosophila *expanded* (*ex*) tumor suppressor [Boedigheimer and Laughon, 1993] and the human neurofibromatosis type 2 tumor suppressor, otherwise known

Abbreviations used: DHR, discs-large homologous region; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; PTPase, protein-tyrosine phosphatase; RT, room temperature; SDS, sodium dodecyl sulphate.

Received February 2, 1995; accepted April 27, 1995.

Address reprint requests to Dr. Wiljan Hendriks, Department of Cell Biology and Histology, University of Nijmegen, Adelbertusplein 1, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

as merlin [Trofatter et al., 1993] or schwannomin [Rouleau et al., 1993]. Both proteins were found to be members of the family of band-4.1-like proteins [Boedigheimer et al., 1993], which mediate the contact of integral membrane proteins with the actin cytoskeleton [Luna and Hitt, 1992]. It has been postulated, therefore, that merlin/schwannomin and *ex* may be involved in membrane-cytoskeletal organization and the control of cell shape, locomotion, and, perhaps, responses to external stimuli [Boedigheimer et al., 1993; Algrain et al., 1993; Tsukita et al., 1993]. Finally, recent observations have implicated cadherin-catenin complexes in neoplasia. Loss of cadherin function results in increased metastatic potential of certain tumors [van Roy and Mareel, 1992]. The tumor suppressor gene APC (adenomatous polyposis coli) associates with catenins and perhaps can modulate cadherin-catenin interaction [Rubinfeld et al., 1993; Su et al., 1993].

Here, we describe the isolation of a cDNA encoding a murine protein-tyrosine phosphatase (PTPase), PTP-BL, that displays intriguing homologies with the neurofibromatosis 2 and Drosophila *dlg* and *ex* proteins. The PTP-BL mRNA is expressed predominantly in epithelial cells, suggesting an important role for PTP-BL in cellular processes that depend on membrane-cytoskeletal interactions, by binding and/or dephosphorylating specific submembranous proteins.

MATERIALS AND METHODS

Isolation of mPTP14 cDNAs

The mPTP14 PCR fragment [Hendriks et al., 1995] was isolated, labelled radioactively by random priming [Feinberg and Vogelstein, 1983], and used to screen a mouse brain λ-ZAPII cDNA phage library (Stratagene, La Jolla, CA). To obtain larger clones, a 5′ fragment of clone mPTP14-5 was used to screen a mouse skin λ-ZAPII cDNA phage library (Stratagene). Hybridization conditions were those of Church and Gilbert [1984]. Washing at high stringency (0.1% SDS, 0.04 M sodium phosphate buffer/pH = 7.4, 1 mM EDTA) was performed three times at 65°C for 20 min. Autoradiography was on Kodak X-omat S1 films at −70°C for 1–2 days using Dupont Cronex (Dupont, Wilmington, DE) intensifying screens. Positive phages were plaque-purified and inserts were rescued as pBluescript SK plasmids according to the manufacturer's protocols.

Clone mPTP14-1.3 was generated by RT-PCR. The sense primer (5′-GGCTT CAT-CATTTCTCCATG-3′), corresponding to position 149–168 of the PTP-BL cDNA sequence, was designed on the basis of the human [Maekawa et al., 1994] and bovine (Walton et al., personal communication) homologous sequence. The antisense primer (5′-CCTGATTGAT-CAAGTTGCC-3′) was chosen close to the 5′ end of clone mPTP14-2 (position 1,390–1,409 of the PTP-BL cDNA sequence). Mouse keratinocyte RNA (2 μg) was isolated and used for cDNA synthesis in a reverse transcription reaction employing random hexamers as described previously [Hendriks et al., 1994]. Following a 5 min incubation at 90°C, 5 μl of the single-stranded cDNA preparation was used as template for PCR. Primers (final concentration 1 ng/μl) were added to a 50 μl reaction mixture containing 20 mM Tris-HCl (pH = 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% BSA, all four dNTPs (each at 250 μM), 1 U *Taq* DNA Polymerase (Perkin-Elmer Cetus, Norwalk, CT), and template material. Thirty-five cycles were performed on a Perkin-Elmer thermal cycler; each cycle involved incubation at 94°C for 45 s, at 50°C for 45 s, and at 72°C for 1.5 min.

Clone mPTP14-R5 was produced by an anchored PCR method described previously [Deen et al., 1992]. An antisense oligonucleotide (5′-GGCTGGCTTTGAGGTACT-3′; pos. 352–369 in the PTP-BL cDNA sequence) served as primer during reverse transcription of mouse keratinocyte mRNA. A nested antisense oligonucleotide (5′-GGATCTTCTCTACATCTGCG-3′; pos. 286–305) and an oligo(dC) primer were used during amplification as described [Deen et al., 1992]. PCR products were cloned into the *Sma*I site of plasmid pBlueScript as described in Hendriks et al. [1995].

Sequence Analysis

Nucleotide sequences were determined using the DNase shotgun strategy [Lin et al., 1985] in combination with the double-stranded DNA dideoxy sequencing method [Hattori and Sakaki, 1986]. DNA sequence gel readings were recorded, compared, edited, and assembled using the IG-SUITE 5.35 package (Intelligenetics, Inc., Mountain View, CA). Deduced protein sequences were analysed using the GCG package [Devereux et al., 1984] provided by the Dutch CAOS/CAMM Center. Databases (EMBL release 40, GenBank rel. 83, PIR-protein rel. 41.1, and

SWISS-PROT rel. 30) were searched for homologous sequences using the BLAST program [Altschul et al., 1990] and DHR-containing proteins were identified using the pattern (K,R) X{2,4}GL(G,R)X{1,5}GGX {8,20}GXXAX{6,9} (G,N)DXXXX(V,I)N allowing 2 mismatches, and subsequent manual screening. Homologies of sequences aligned with PILEUP and LINEUP were determined using DISTANCES with thresholds of 1.5 and 0.6 for identity and similarity values, respectively [Devereux et al., 1984].

Assay of Phosphatase Activity

pGEX-BL was constructed by ligating the 1.2 kb DNA polymerase-treated (blunt) *Hind*III fragment of mPTP14-2, encoding the last 360 amino acids of PTP-BL, into the *Sma*I site of pGEX-3X [Smith and Johnson, 1988]. *Escherichia coli* DH-5α (Gibco-BRL, Gaithersburg, MD) transformed with pGEX-BL or the empty vector pGEX-3X were grown in 10 ml of Luria broth containing 100 μg/ml ampicillin until A_{550} reached 0.7, and induced with 0.1 mM IPTG for 4 h at 37°C. GST-containing proteins were isolated according to Frangioni and Neel [1993]. Briefly, 1.5 ml of the bacterial culture was pelleted, washed once with 200 μl of cold STE (10 mM Tris, pH 8.0, 150 mM NaCl, 1 mM EDTA), resuspended in 135 μl of STE containing 100 μg/ml of lysozyme, and incubated on ice for 15 min. Then 1.5 μl 0.5 M DTT and 1 μl PMSF (100 mM) were added, and bacteria were lysed by the addition of 24 μl of 10% N-laurylsarcosine in STE. After brief vortexing and 1 min sonication on ice, the lysate was clarified by centrifugation at 10,000*g* for 5 min, at 4°C. The supernatant was transferred to a new tube, and 40 μl of 10% Triton X-100 in STE was added. From the 200 μl lysate, the GST fusion protein was purified over glutathione agarose beads (Sigma, St. Louis, MO) exactly as described [Frangioni and Neel, 1993]. Purified GST or GST-BL protein was then used for tyrosine phosphatase activity determination using the colorimetric substrate p-nitrophenyl phosphate (pNPP). Reactions were performed with lysate amounts and substrate concentrations that showed a constant reaction velocity with respect to time. Each 200 μl of reaction mixture contained 50 mM Imidazole, pH 7.5, 5 mM pNPP, 10 mM DTT, 10 mM EDTA, and 1 μg of protein. Potential modifiers of phosphatase activity were added to some reaction mixtures. Reaction mixtures were incubated for 30 min at 37°C and the reaction was terminated by the addition of 800 μl 0.2 M NaOH. Absorbance was measured at 410 nm. Two phosphotyrosine-containing peptides (the hirudin 53–65 C-terminal fragment and the human gastrin 1–17 N-terminal fragment) were also used in a non-radioactive phosphatase assay as described by the manufacturer (Boehringer Mannheim, Indianapolis, IN).

RNA In Situ Hybridization

Embryos were isolated from pregnant NMRI mice at developmental stages indicated in the text. The day of plug detection was considered to be day 0.5 post conception (p.c.). Embryos and tissue samples were fixed overnight with 4% paraformaldehyde in PBS at 4°C. Cryosections (10 μm) were mounted on 3-aminopropyltriethoxy-silane-coated slides and RNA in situ hybridization was performed as described previously [Bächner et al., 1993]. To generate "sense" and "antisense" RNA probes, the mouse mPTP14-2 cDNA clone in pBlueScript-SK was linearized with *Xho*I or *Not*I and used for in vitro transcription by T3 or T7 RNA Polymerase, respectively. Both probes were labelled with α^{35}S-UTP to a specific activity of $>10^9$ dpm/μg. Probe length was reduced to 150–200 nucleotides by alkaline hydrolysis. The slides were prehybridized at 54°C in a solution containing 50% formamide, 10% dextran sulphate, 0.3 M NaCl, 10 mM Tris, 10 mM sodium phosphate (pH 6.8), 20 mM DTT, 0.2 × Denhardt's reagent, 0.1% Triton X-100, 0.1 mg/ml *E. coli* RNA, and "cold" 0.1 mM αS-UTP. For hybridization, 80,000 dpm/μl α^{35}S-UTP labelled RNA probe was added to the hybridization mix and the hybridization was continued at 54°C for 16 h in a humid chamber. Subsequently, the slides were washed in hybridization salt solution to which 20 mM dithiothreitol was added. After RNase A digestion, slides were washed for 30 min at 37°C with 2 × SSC, 0.1% SDS, and 30 min with 0.1 × SSC, 0.1% SDS, and dehydrated by increasing concentrations of ethanol. The slides were coated with Ilford K5 photoemulsion for autoradiography. After 14–21 days of exposure at 4°C, the slides were developed in Kodak D19b and stained with Giemsa. The sections were analyzed with bright- and dark-field illumination using a Zeiss (Thornwood, NY) SV8 stereo-microscope and an Axiophot microscope and photographed using Agfa Ortho black-and-white film.

RESULTS

Cloning of mPTP14 cDNAs

We recently isolated murine PTPase cDNA fragments [Hendriks et al., 1995] using degenerate primers based on conserved regions in the rapidly expanding PTPase gene family [Walton and Dixon, 1993]. One of these fragments, mPTP14, showed the highest homology with the PTPases MEG [Gu et al., 1991] and PTPH1 [Yang and Tonks, 1991] (29 and 31% identity at the amino acid level, respectively). MEG and PTPH1 contain one catalytic phosphatase domain and an amino-terminal stretch with significant similarity to the band 4.1-type protein family. However, the length of the mPTP14 transcript (8 kb) that was detectable in various murine tissues [Hendriks et al., 1995] was significantly larger than those reported for MEG and PTPH1 (around 4 kb), and was therefore expected to encode a much larger protein product. To determine the nature of the additional sequence motifs present in the large mPTP14 transcript, we set out to clone the corresponding cDNA.

Screening of a mouse brain cDNA library yielded several positive clones, among which mPTP14-5 was the largest with an insert length of 4.5 kbp. A murine skin cDNA library was screened to obtain longer clones, since strong signals were detected by the mPTP14 probe on Northern blots containing keratinocyte RNA [Hendriks et al., 1995]. The longest cDNA identified, mPTP14-2, spanned 6.5 kbp and its DNA sequence was determined. As expected from the length of the corresponding transcript, mPTP14-2 does not represent a full-length clone. While this work was nearing completion, the sequence of human PTP-BAS [Maekawa et al., 1994] was published that exhibited high homology with our clones. A bovine homolog has also been reported [Mauro and Dixon, 1994]. By comparing the human and bovine (kindly provided by Drs. K. Walton, Q. Vega, and J. Dixon) nucleotide sequences, we were able to design an oligonucleotide primer close to the 5′ end of the open reading frame. Using this primer in combination with an antisense primer from the 5′ region of clone mPTP14-2, a 1.3 kbp cDNA fragment (mPTP14-1.3) was amplified by RT-PCR from mouse keratinocyte RNA. Subsequently, using the mPTP14-1.3 sequence data, antisense oligonucleotide primers were designed to clone additional 5′ sequences by anchored PCR. In this way, a nearly full-length cDNA sequence for the mPTP14 transcript was assembled from mPTP14-2, mPTP14-1.3, and mPTP14-R5 (Fig. 1A). The 7,922 bp sequence spans 7 nucleotides of the putative 5′ untranslated region, the complete open reading frame and the poly-(A) addition signal-containing 3′ untranslated region (acc. no. Z32740).

Analysis of the Encoded PTP-BL Protein

The 2,460 amino acid protein that is predicted by the mPTP14 open reading frame (Fig. 1B) displays 80% identity and 86% similarity to the human PTP-BAS type 1 sequence [Maekawa et al., 1994], and we therefore propose the name PTP-BL (BAS-like) for the mPTP14-encoded protein. Recent reports have also described the cloning of PTPL1 [Saras et al., 1994] and hPTP1E [Banville et al., 1994], which are actually splice variants of PTP-BAS. Like its human homologs, PTP-BL exhibits intriguing sequence features (Fig. 1). As was anticipated from its homology to PTPH1 and MEG, PTP-BL contains a tyrosine phosphatase domain (residues 2,181–2,431) and a band 4.1-like sequence (aa 577–872), which is regarded as being a plasma membrane binding sequence that serves as a molecular link between integral membrane proteins and the cytoskeleton [Luna and Hitt, 1992]. In between the band 4.1-like sequence and the

Fig. 1. **(appears on page 422)** **A:** Schematic representation of overlapping mPTP14 cDNA clones. cDNA clone names are on the left and their relative positions are shown by thick black bars. The scale indicates nucleotide positions. A restriction map of the composite cDNA is drawn. B, *Bgl*II; H, *Hind*III; S, *Sma*I. The coding region, resulting in PTP-BL protein, is diagrammed schematically. The band 4.1-like domain (*hatched bar*), five DHR motifs (*circles*), and the protein tyrosine phosphatase domain (*black box*) are indicated. **B:** Alignment of the deduced amino acid sequences of mouse PTP-BL (acc. no. Z32740) and human PTP-BAS type 1 (acc. no. D21209). Identical residues are represented by dashes in the human sequence and dots indicate gap positions that were introduced. Amino acid residue numbers are indicated on the right. The band 4.1-like domain is boxed with a single line, the five DHR motifs are depicted on a gray background, and the catalytic domain is indicated using a double-lined open box. The part represented in clone mPTP14 is underlined. Protein regions that are not present in certain human isoforms due to alternative splicing [Maekawa et al., 1994; Saras et al., 1994; Banville et al., 1994] are underlined using small open circles. Two putative tyrosine kinase phosphorylation sites are indicated by black triangles below the human and above the mouse tyrosine residues. Black squares below the PTP-BAS sequence indicate the leucine and methionine residues that make up the leucine zipper motif [Saras et al., 1994].

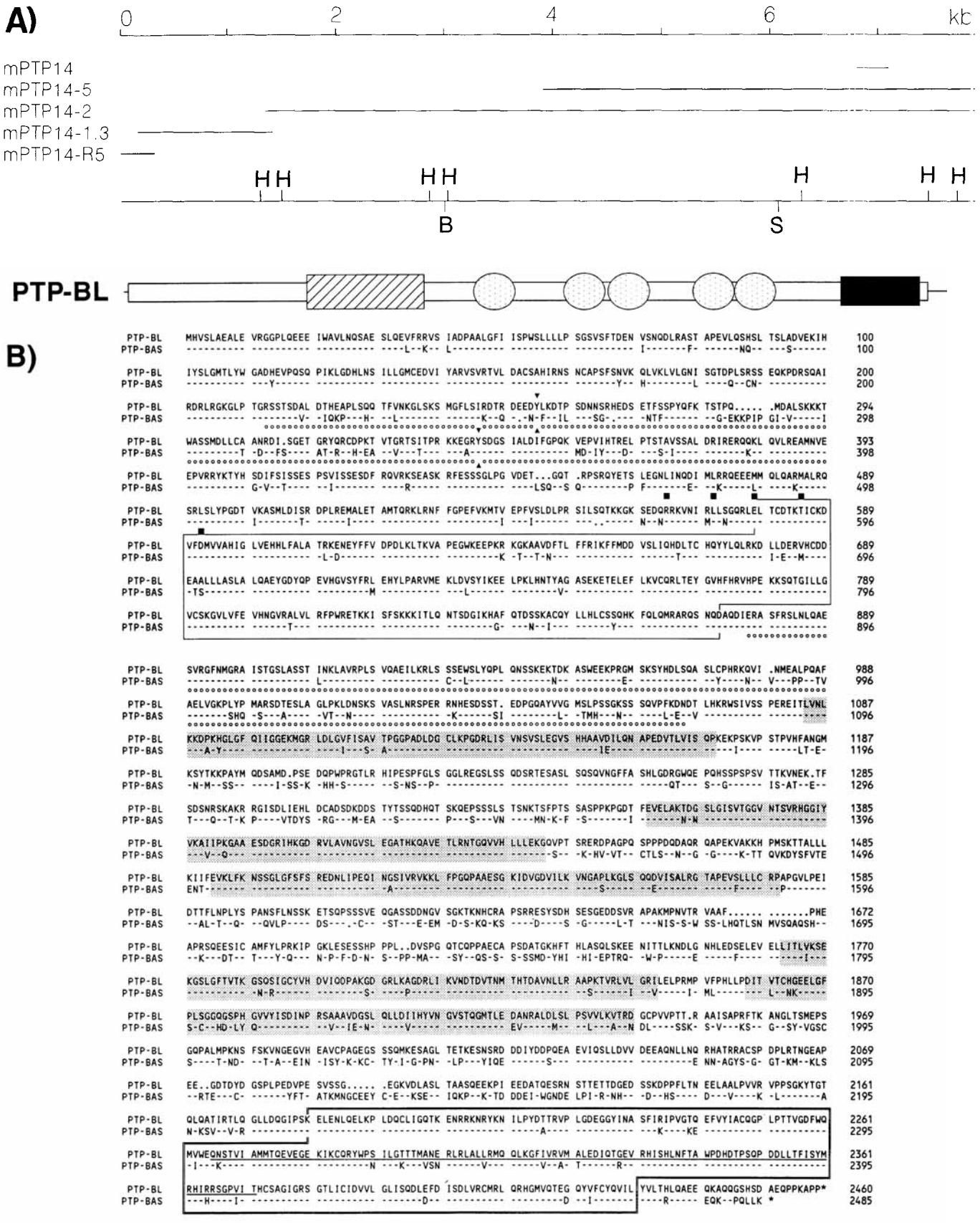

Figure 1 (legend on page 421)

catalytic phosphatase domain, five 80-amino acid repeat regions were found that are known as GLGF motifs [Cho et al., 1992] or discs-large homologous regions (DHR) [Bryant et al., 1993; Woods and Bryant, 1993] (Fig. 1). The DHR motif was first detected in a family of membrane-associated guanylate kinases that are localized at specialized junctional structures or junction-like areas [Bryant et al., 1993; Woods and Bryant, 1993]. It has been speculated that the DHR motif mediates coiled-coil interactions with the actin cytoskeleton [Woods and Bryant, 1991] or that it is capable of regulating guanylate kinase enzyme activity [Cho et al., 1992]. Over the past year several other proteins have been reported to contain DHR motifs, including the PTPases PTP-BAS/PTPL1/hPTP1E, MEG, and PTPH1 [Maekawa et al., 1994; Saras et al., 1994; Banville et al., 1994; Theisen et al., 1994; Prasad et al., 1993; Lue et al., 1994]. Experiments to establish a possible function for the DHR motif have been limited to the observation that protein 4.1 can bind to the DHR-containing region of the human homolog of DlgA in vitro [Lue et al., 1994]. No homologies other than to the human PTP-BAS isoforms were found for the N-terminal segment of PTP-BL in the databases.

Comparison of PTP-BL to Its Human Homolog

The availability of both human and murine sequences for this unusually large non-receptor-type PTPase has allowed an interspecies comparison (Fig. 1; Table I). As outlined above, the PTP-BL sequence can be divided into several domains. The evolutionary conservation of these domains has been determined and amino acid identities and similarities for the different segments are listed in Table I. For the band 4.1-like sequence, the five DHR motifs and the single phosphatase domain, 90% or more identity (94–96% similarity) is observed between the human and mouse sequences. The spacer regions surrounding the DHR motifs are only 64% identical (73% similar), indicating reduced structural or functional constraints within these segments. The N-terminal segment, which lacks obvious homologies to other proteins in the databases, displays higher values, of 86% identity and 92% similarity, respectively (Table I). A more careful scrutiny of the murine and human N-terminal sequences (Fig. 1B) shows them to comprise three regions. Regions 1 and 3 yield similarity scores as high as those of the other conserved protein domains described above. In contrast, region 2 (corresponding to residues 213–393 of PTP-BL) is less well conserved and is spliced out in some hPTP1E variants. Interestingly, this region contains two conserved potential tyrosine kinase phosphorylation sites at residues 255 and 339, which suggests a regulatory role for this module in the stability, activity, or subcellular localization of the protein. A putative leucine zipper motif has been identified within the third part of the N-terminal domain [Saras et al., 1994] which may explain the high degree of similarity between the mouse and human sequences in this region of PTP-BL. However, the third leucine residue present in the human zipper motif has been replaced by a methionine in the mouse sequence (residue 478). Nevertheless, a significant potential to form coiled coil structures can be predicted for the third

TABLE I. Sequence Comparison Between the Various Domains as Present in Mouse PTP-BL and Human PTP-BAS Type 1

Protein segment	PTP-BL residues	Identity (%)	Similarity (%)
N-terminal segment	1–576	86	92
Part 1	1–212	93	96
Part 2	213–393	75	84
Part 3	394–576	88	96
Band 4.1-like domain	577–872	93	96
DHR-spanning part	873–2,180	73	80
DHR motifs only	446 in total	90	94
Spacer regions	862 in total	64	73
Catalytic domain	2,181–2,431	92	95
Total protein	1–2,460	80	86

TABLE II. Phosphotyrosine Phosphatase Activity of GST-BL Fusion Protein in the Presence or Absence of Various Potential Inhibitors*

Protein	Modifier	Activity (% of GST-BL)
GST	—	<1
GST-BL	—	100
GST-BL	0.5 mM vanadate	98
GST-BL	2.5 mM vanadate	91
GST-BL	0.1 mM phenylarsine oxide	49
GST-BL	1.0 μM okadaic acid	103
GST-BL	0.1 mM $ZnCl_2$	102

*All assays were performed in triplicate. An activity of 100% corresponds to an A_{410} of 0.347 after a 30 min incubation, as outlined in Materials and Methods.

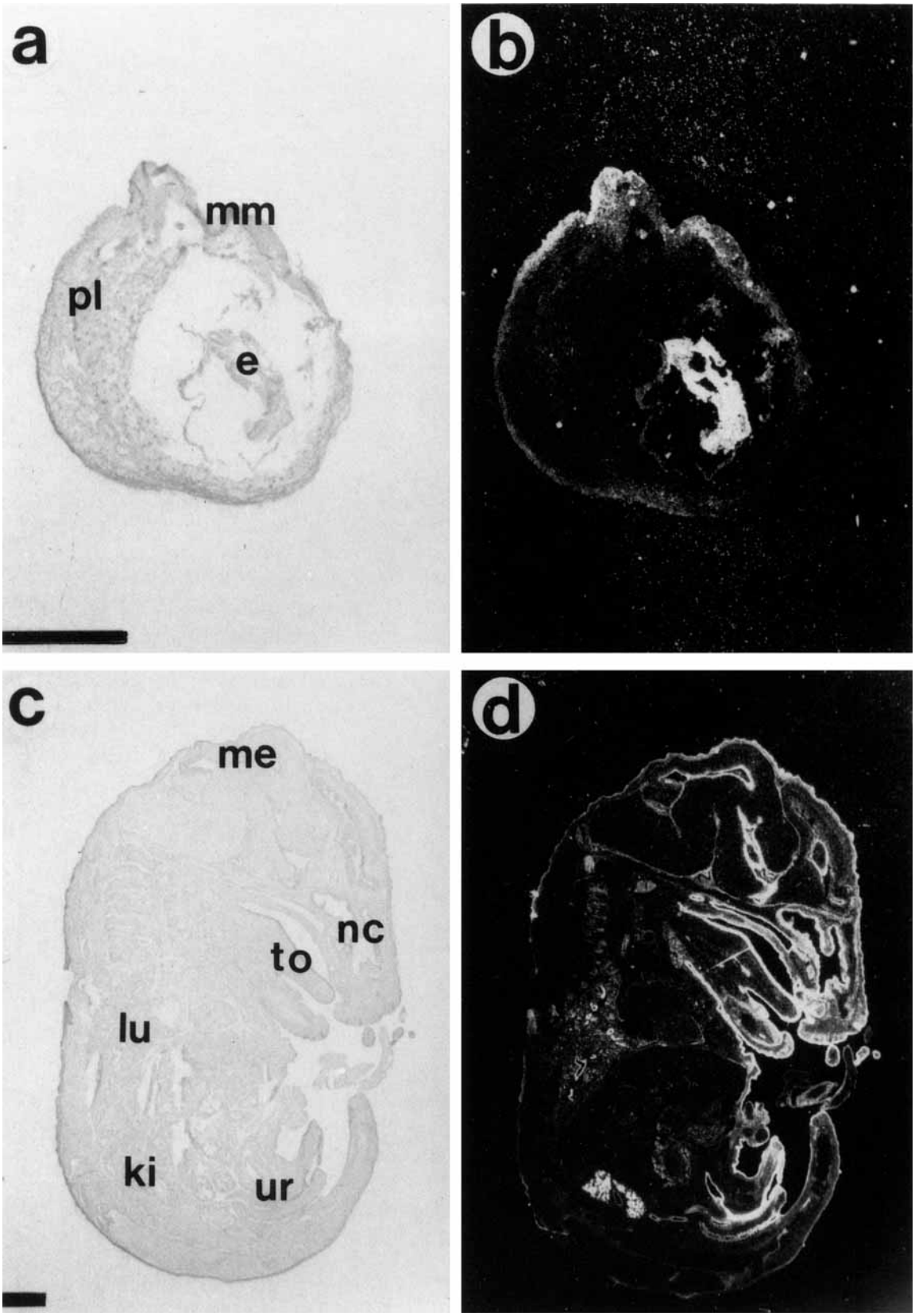

Fig. 2. Expression pattern of PTP-BL during mouse embryogenesis. Bright (**a,c**) and dark field (**b,d**) recordings of parasaggital sections hybridized with the antisense PTP-BL riboprobe are shown. A sense control hybridization did not result in a specific signal (data not shown). **a,b:** High expression of PTP-BL in all cells of a 9.5-day-old embryo is shown. No specific expression is found in the placenta. **c,d:** The restriction of the PTP-BL expression to epithelial cells of a 16.5-day-old embryo is visible. e, embryo; ki, kidney; lu, lung; me, metencephalon; mm, mesometrium; nc, nasal cavity; pl, placenta; to, tongue; ur, ureter. The bar represents 1 mm.

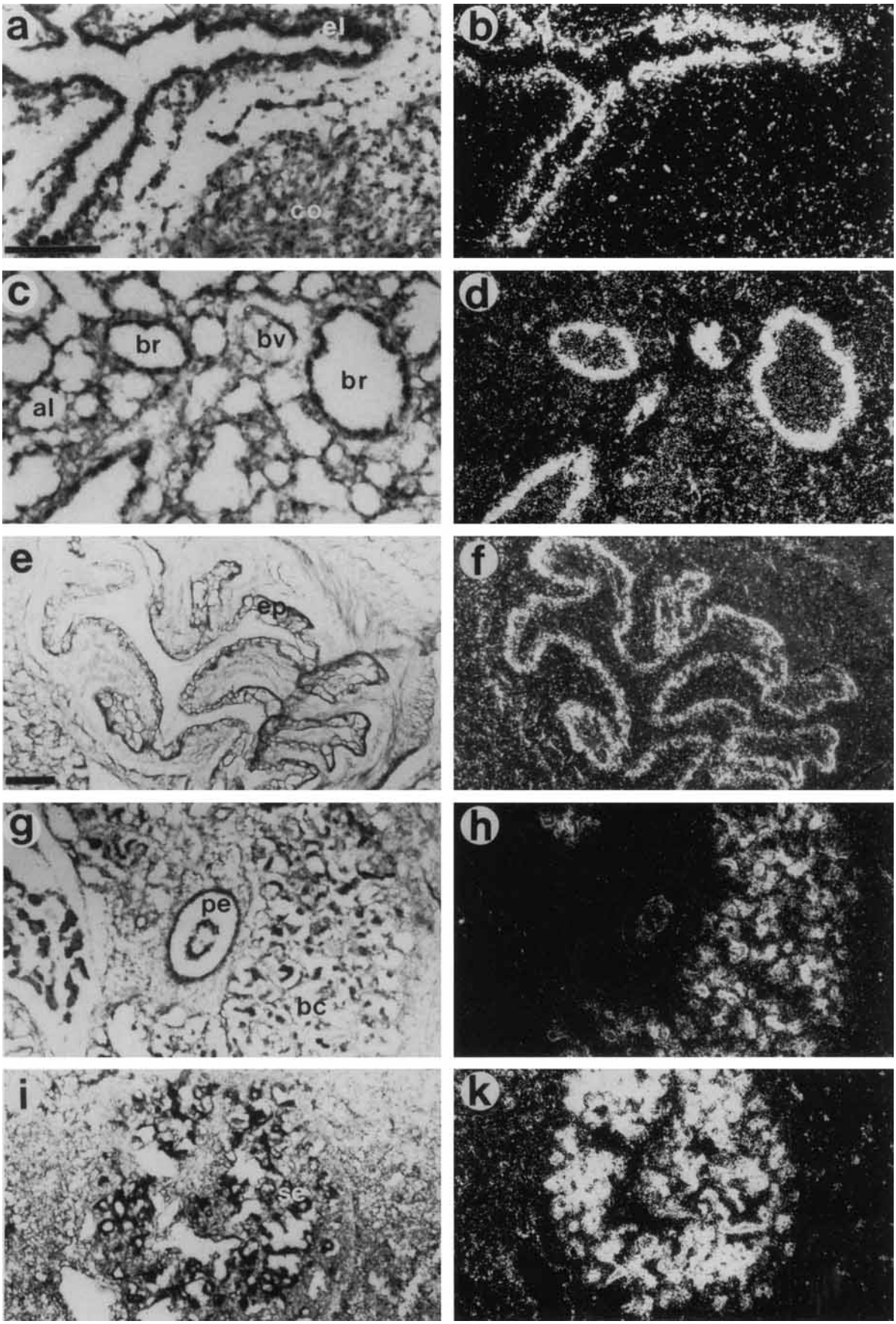

Figure 3 (legend on page 426)

N-terminal segment in both the mouse and the human sequence [Lupas et al., 1991], in sharp contrast to the leucine heptad repeat that is found at the end of the phosphatase domain (residues 2,384–2,405). It remains to be investigated, however, whether the putative zipper region can indeed mediate di- or trimerization of the protein.

Phosphatase Activity of PTP-BL

A single conserved tyrosine phosphatase catalytic domain, which contains the highly conserved sequence VHCxAGxxR, is found at the C-terminus of PTP-BL. To demonstrate that PTP-BL indeed exhibits tyrosine phosphatase activity, a 1.2 kb *Hind*III fragment (Fig. 1A) encoding the last 360 residues was cloned into the bacterial expression vector pGEX-3X [Smith and Johnson, 1988]. The predicted 65 kDa recombinant GST fusion protein was detected on SDS/PAGE when transformed bacteria were grown in the presence of IPTG (data not shown). The fusion protein was present in the particulate fraction, probably due to the formation of inclusion bodies. Using a recently described sarkosyl method [Frangioni and Neel, 1993], the fusion protein was solubilized and purified over glutathione agarose beads. In spite of the presence of protease inhibitors, a considerable portion of the GST fusion protein appeared to be degraded. Since the extent of degradation could not be influenced by changing the experimental conditions, we surmise that the recombinant protein was susceptible to protease attack in vivo. Nevertheless, the recombinant GST-BL protein, but not GST alone, displayed significant phosphatase activity using pNPP as a substrate (Table I). The observed activity appeared sensitive to the tyrosine phosphatase inhibitor phenylarsine oxide (PAO). Under the conditions used, phosphatase activity was negligibly influenced by vanadate and was not modulated by Zn^{2+}, nor by the serine/threonine phosphatase inhibitor okadaic acid (Table II). Furthermore, two different phosphotyrosine-containing peptides were dephosphorylated by the GST-BL protein as determined in a non-radioactive assay (results not shown), clearly demonstrating phosphotyrosine phosphatase activity for PTP-BL.

PTP-BL Is Expressed in Epithelia

Preliminary Northern blot analyses had shown PTP-BL to be expressed at low levels in many different tissues [Hendriks et al., 1995]. In addition, RT-PCR experiments had revealed the expression of PTP-BL mRNA in murine embryonic stem cells derived from the blastocyst inner cell mass [Hendriks et al., 1994]. Therefore, we performed RNA in situ hybridization experiments on fetal and postnatal murine tissue sections to determine the expression patterns during development and in adult tissues more precisely. As demonstrated in Figures 2 and 3, hybridization with the PTP14-2 antisense riboprobe reveals distinct spatial and temporal expression patterns. In the early development PTP-BL is expressed at high levels throughout the embryo (Fig. 2a,b). During subsequent development, expression becomes restricted to epithelial cell lineages, e.g., epithelia of the skin and the oesophagus, the epithelia surrounding the vesicles of the brain, the epithelia of the nasal cavity, the lung, the kidney, the ureter, and the bladder (Fig. 2c,d). In late development high expression is found, e.g., in the ependymal cell layer, which forms the epithelia surrounding the ventricles in brain (Fig. 3a,b), the epithelia of bronchioli in the lung (Fig. 3c,d), and the epithelia of the stomach (Fig. 3e,f). High expression is also evident in cells of the Bowman's capsules of the kidney (Fig. 3g,h) and the seminiferous epithelia of the testis (Fig. 3i,k). There is only low expression in epithelial cells of the gut. Expression was also detected in some non-epithelial cells, such as the connective tissue surrounding the vertebrae in day 14.5 p.c. embryos (data not shown). No expression was observed in neuronal cells, muscle, or liver.

DISCUSSION

We have isolated cDNAs encoding a novel murine cytoplasmic-type protein-tyrosine phos-

Fig. 3. (appears on page 425) Expression of PTP-BL in late development. Bright field (**a,c,e,g,i**) and dark field (**b,d,f,h,k**) images are shown of different tissue sections of 18.5-day-old mouse embryos hybridized with the antisense PTP-BL riboprobe. A sense control hybridization did not yield a specific signal (data not shown). Expression is restricted to different types of cells of the epithelial lineage. **a,b:** The high expression within the ependymal cell layer of the fourth ventricle of the brain is visible. **c,d:** The high expression in the epithelia of the bronchioli of the long and **(e,f)** the high expression in the epithelia of the stomach is shown. **g,h:** The expression in the cells forming the Bowman's capsules of the kidney. **i,k:** The expression within the seminiferous epithelia of the testis. al, alveola; bc, Bowman's capsule; br, bronchiole; bv, blood vessel; co, cochlea; el, ependymal cell layer; ep, epithelia; pe, pelvis; se, seminiferous tubule. The bar represents 100 μm.

phatase, PTP-BL, from brain and skin cDNA libraries. Using RNA in situ hybridization, PTP-BL expression was studied in a developmental perspective. PTP-BL mRNA expression is already detectable at very early stages of development, e.g., in embryonic stem cells [Hendriks et al., 1994] and in all cells of a 9.5-day-old embryo (Fig. 2). In late stages of development PTP-BL expression could be demonstrated in epithelial cell layers, most notably in the skin, nasal cavity, oesophagus, stomach, kidney, and brain. This is in keeping with the expression pattern for the human gene as judged from Northern analysis. PTP-BAS expression was found in a wide variety of human tissues and a comparison of fetal and adult expression patterns points to a higher expression level in fetal brain for the PTP-BAS transcript [Maekawa et al., 1994]. This observation may be due to the relative prominence of the brain cavities, and thus ependymal cell layers, during the early embryonic stages compared to adult brain.

The deduced protein sequence for PTP-BL displays an 80% sequence homology with the recently published human PTPases PTP-BAS/PTPL1/hPTP1E [Maekawa et al., 1994; Saras et al., 1994; Banville et al., 1994]. The N-terminal segment of PTP-BL exhibits no apparent homology to other known proteins, but does contain two putative tyrosine kinase phosphorylation sites and a leucine zipper motif with coiled coil-forming potential. PTP-BL also contains a band 4.1-like domain, five DHR motifs, and a single protein-tyrosine phosphatase catalytic domain (Fig. 1) suggesting a membrane/cytoskeletal localization for this PTPase. Five possible PEST domains surrounding the fourth and fifth DHR motif have been reported [Banville et al., 1994], suggesting that PTP-BAS might be rapidly degraded within the cell. In general, however, cell cortical and cytoskeletal proteins are rather stable. In line with this view, preliminary pulse-chase experiments on cells transfected with expression constructs containing the *Bgl*II-*Sma*I fragment spanning the PTP-BL DHR region (Fig. 1B) point to a half-life of more than 16 h for this segment (Hendriks et al., unpublished data). This period is much longer than that observed for genuine PEST-containing proteins [Rechsteiner et al., 1987].

Over the past year several proteins other than protein-tyrosine phosphatases and membrane-associated gyanylate kinase homologues have been found to contain DHR motifs [Banville et al., 1994; Maekawa et al., 1994; Lue et al., 1994; Prasad et al., 1993; Saras et al., 1994; Theisen et al., 1994]. Most of these proteins contain a single DHR motif, such as LCF [Cruikshank et al., 1994], syntrophins [Adams et al., 1993b], AF-6 [Prasad et al., 1993], *dishevelled* [Theisen et al., 1994], X11 [Duclos et al., 1993], and the product from an altered *ros*1 transcript [Sharma et al., 1989]. Interestingly, lymphocyte chemoattractant factor (LCF) is a novel type of interleukin that consists almost entirely of a single DHR motif and whose biological activity depends on autoaggregation [Cruikshank et al., 1994], suggesting that DHR motifs mediate homophilic interactions. As mentioned previously, the DHR region of hdlg can bind to protein 4.1 in vitro [Lue et al., 1994], implying that *heterophilic* DHR-mediated interactions can occur as well. Association to band 4.1 has also been observed for the DHR-containing protein p55 [Marfatia et al., 1994]. Syntrophins are dystrophin-associated proteins located at the cell cortex [Adams et al., 1993b] but it is not known whether their DHR motifs mediate this interaction. Genetic evidence suggests that the Drosophila protein *dishevelled* participates in signalling events downstream of *wingless* that occur close to the membrane [Theisen et al., 1994]. Like DlgA, *dishevelled* is required to establish cell polarity and it is tempting to speculate that the DHR motif determines the cell cortical localization required for its function. The human X11 protein contains two DHR motifs at its C-terminus. This protein is a candidate for Friedreich Ataxia (FRDA), an autosomal recessive degenerative disorder affecting both central and peripheral nervous systems [Duclos et al., 1993]. A crucial role for X11 as part of the membrane skeleton in establishing polarity of neuronal cells would be in line with the observed FRDA phenotype.

To obtain more clues about potential roles for DHR motifs we performed homology and pattern searches using the latest database releases, and five additional sequences were found to contain a DHR motif (Fig. 4). Surprisingly, one of these proteins, X104, is the product of another gene which resides within the FRDA gene region, but which has been excluded as a candidate for the disease [Duclos et al., 1994]. X104 is in fact the human homolog of canine ZO-2 [Jesaitis and Goodenough, 1994], a ubiquitous component of the epithelial tight junction complex, and was found to contain three DHR motifs (Fig. 4). Two expressed sequence tags, T06631

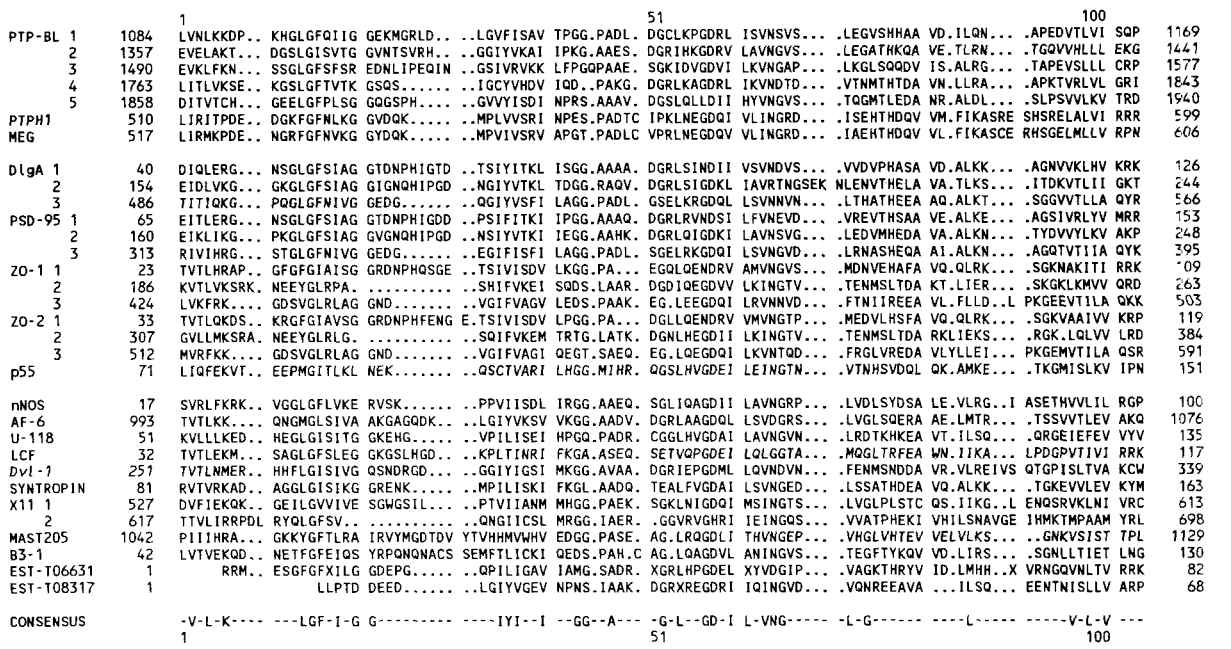

Fig. 4. Multiple sequence alignment of DHR motifs from various proteins. Black dots indicate gap positions and a deduced consensus sequence is shown at the bottom. Protein segments that were used are flanking the motif sequences. Motifs are numbered starting from the N-terminus of the corresponding protein. The following sequences were used: mouse PTP-BL (acc. no. Z32740); human PTPH1 (P26045) and MEG (P29074), Drosophila DlgA (P31007), rat PSD-95 (P31016), mouse ZO-1 (D14340), human ZO-2 (L27476), p55 (Q00013), nNOS (P29475), AF-6 (U02478), U-118 (X51619) and LCF (M90391), mouse Dvl-1 (U10115) and syntrophin-1 (U00677), human X11 (A47176), mouse MAST205 (U02313), human B3-1 (S43424), and EST sequences (T06631 and T08317). The obtained alignments were all judged as significant using a sophisticated shuffling method derived from program RDF2 [Pearson and Lipman, 1988].

and T08317 [Adams et al., 1993a], also harboured DHR-like sequences in their open reading frames. In addition, the N-terminal half of the small leucine zipper-containing protein B3-1 [Dixon et al., 1993] is comprised of a DHR motif. Finally, and most interestingly, we identified a single DHR motif in the testis-specific serine/threonine kinase MAST205 [Walden and Cowan, 1993]. MAST205 can associate to MAP-coated microtubules and this binding is displayed by a small protein segment just downstream of the kinase domain. This downstream segment displays 20–23% sequence identity to the DHR motif prototypes of DlgA (Fig. 4). Since the segment does not bind to MAP-free tubulin, these data suggest that DHR motifs may enable interactions with proteins that decorate tubulin filaments. Indeed immunoprecipitation of MAST205 coprecipitates a 75 kDa protein [Walden and Cowan, 1993]. It is noteworthy to mention that a 78 kDa component was also detected in precipitates of the human homolog of PTP-BL, PTPL1 [Saras et al., 1994]. In conclusion, evidence is accumulating that the DHR motif is a protein-binding interface that can mediate assembly into submembranous and cytoskeletal structures.

The mRNA expression pattern and the sequence homologies in PTP-BL imply that its substrates will be found among the proteins located at the cell cortex of epithelial cells. Many submembranous proteins contain phosphotyrosyl residues and protein phosphorylation has indeed been implicated in the regulation of structural changes in the membrane skeleton and cytoskeleton [Luna and Hitt, 1992]. The cloning of the neurofibromatosis type 2, *dlg*, and *ex* tumor suppressor genes has demonstrated the tremendous impact of peripheral membrane proteins on cell growth and differentiation [Algrain et al., 1993; Boedigheimer et al., 1993; Bryant et al., 1993; Tsukita et al., 1993]. Further investigations of the biological properties of PTP-BL are therefore expected to yield more information on the interplay of membrane-cytoskeletal protein complexes, both in normal and malignant cells.

NOTE ADDED IN PROOF

After submission of this manuscript an almost identical PTPase sequence was described:

Chida D, Kume T, Mukouyama Y, Tabate S, Nomura N, Thomas ML, Watanabe T, Oishi M (1995): Characterization of a protein tyrosine phosphatase (RIP) expressed at a very early stage of differentiation in both mouse erythroleukemia and embryonal carcinoma cells. FEBS Lett 358:233–239. Also, an extended overview of DHR-containing proteins has appeared recently: Ponting CP, Phillips C (1995): DHR domains in syntrophins, neuronal NO synthases and other intracellular proteins. Trends Bioch Sci 20:102–103.

ACKNOWLEDGMENTS

We thank Joost Schalkwijk for mouse keratinocytes and the murine skin cDNA library, and Kevin Walton, Quinn Vega, and Jack Dixon for sharing information prior to publication. We also thank Peter Deen for helpful advice on anchored-PCR, and Dave Iles for critically reading the manuscript. The use of the services and facilities of the Dutch CAOS/CAMM Center is gratefully acknowledged. This work is supported in part by a grant from the Dutch Cancer Society (W.H.). D.B. and H.H. are supported by grants of the Deutsche Forschungsgemeinshaft, and U.Z. is supported by a grant of the Land Baden-Württemberg.

REFERENCES

Adams MD, Soares MB, Kerlavage AR, Fields C, Venter JC (1993a): Rapid cDNA sequencing (expressed sequence tags) from a directionally cloned human infant brain cDNA library. Nature Genet 4:373–380.

Adams ME, Butler MH, Dwyer RM, Peters MF, Murnane AA, Froehner SC (1993b): Two forms of mouse syntrophin, a 58 kd dystrophin-associated protein, differ in primary structure and tissue distribution. Neuron 11:531–540.

Algrain M, Arpin M, Louvard D (1993) Wizardry at the cell cortex. Curr Biol 3:451–454.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990): Basic local alignment search tool. J Mol Biol 215:403–410.

Bächner D, Manca A, Steinbach P, Wöhrle D, Just W, Vogel W, Hameister H, Poustka A (1993): Enhanced expression of the murine FMR1 gene during germ cell proliferation suggests a special function in both the male and the female gonad. Hum Mol Genet 2:2043–2050.

Banville D, Ahmad S, Stocco R, Shen S-H (1994): A novel protein-tyrosine phosphatase with homology to both the cytoskeletal proteins of the band 4.1 family and junction-associated guanylate kinases. J Biol Chem 269:22320–22327.

Boedigheimer M, Laughon A (1993): Expanded, a gene involved in the control of cell proliferation in imaginal discs. Development 118:1291–1301.

Boedigheimer M, Bryant P, Laughon A (1993): Expanded, a negative regulator of cell proliferation in Drosophila, shows homology to the NF2 tumor suppressor. Mech Dev 44: 83–84.

Bryant PJ, Watson KL, Justice RW, Woods DF (1993): Tumor suppressor genes encoding proteins required for cell interactions and signal transduction in Drosophila. Development (Suppl. 1993):239–249.

Cho K-O, Hunt CA, Kennedy MB (1992) The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein. Neuron 9:929–942.

Church GM, Gilbert W (1984): Genomic sequencing. Proc Natl Acad Sci USA 81:1991–1995.

Cruikshank WW, Center DM, Nisar N, Wu M, Natke B, Theodore AC, Kornfeld H (1994): Molecular and functional analysis of a lymphocyte chemoattractant factor: Association of biologic function with CD4 expression. Proc Natl Acad Sci USA 91:5109–5113.

Deen PM, Dempster JA, Wieringa B, van Os CH (1992): Isolation of a cDNA for rat CHIP28 water channel: High mRNA expression in kidney cortex and inner medulla. Biochem Biophys Res Commun 188:1267–1273.

Devereux J, Haeberli P, Smithies O (1984): A comprehensive set of sequence analysis programs for the VAX. Nucleic Acid Res 12:387–395.

Dixon B, Sahely B, Liu L, Pohajdak B (1993): Cloning of a cDNA from human NK/T cells which codes for an unusual leucine zipper containing protein. Biochim Biophys Acta 1216:321–324.

Duclos F, Boschert U, Sirugo G, Mandel J-L, Hen R, Koenig M (1993): Gene in the region of the Friedreich Ataxia locus encodes a putative transmembrane protein expressed in the nervous system. Proc Natl Acad Sci USA 90:109–113.

Duclos F, Rodius F, Wrogemann K, Mandel J-L, Koenig M (1994): The Friedreich Ataxia region: Characterization of two novel genes and reduction of the critical region to 300 kb. Hum Mol Genet 3:909–914.

Feinberg AP, Vogelstein B (1983): A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13.

Frangioni JV, Neel BG (1993): Solubilization and purification of enzymatically active glutathione S-transferase (pGEX) fusion proteins. Anal Biochem 210:179–187.

Gu M, York JD, Warshawsky I, Majerus PW (1991): Identification, cloning, and expression of a cytosolic megakaryocyte protein-tyrosine phosphatase with sequence homology to cytoskeletal protein 4.1. Proc Natl Acad Sci USA 88:5867–5871.

Hattori M, Sakaki Y (1986): Dideoxy sequencing method using denatured plasmid templates. Anal Biochem 152: 232–238.

Hedrick L, Cho KR, Vogelstein B (1993): Cell adhesion molecules as tumour suppressors. Trends Cell Biol 3:36–39.

Hendriks W, Brugman C, Schepens J, Wieringa B (1994): Rapid assessment of protein-tyrosine phosphatase expression levels by RT-PCR with degenerate primers. Mol Biol Rep 19:105–108.

Hendriks W, Schepens J, Brugman C, Zeeuwen P, Wieringa B (1995): A novel receptor-type protein tyrosine phosphatase with a single catalytic domain is specifically expressed in mouse brain. Biochem J 305:499–504.

Jesaitis LA, Goodenough DA (1994): Molecular characterization and tissue distribution of ZO-2, a tight junction protein homologous to ZO-1 and the Drosophila discs-large tumor suppressor protein. J Cell Biol 124:949–961.

Lin H-C, Lei S-P, Wilcox G (1985): An improved DNA sequencing strategy. Anal Biochem 210:179–187.

Lue RA, Marfatia SM, Branton D, Chishti AH (1994): Cloning and characterization of hdlg: The human homologue of the Drosophila discs large tumor suppressor binds to protein 4.1. Proc Natl Acad Sci USA 91:9818–9822.

Luna EJ, Hitt AL (1992): Cytoskeleton-plasma membrane interactions. Science 258:955–964.

Lupas A, van Dyke M, Stock J (1991): Predicting coiled coils from protein sequences. Science 252:1162–1164.

Maekawa K, Imagawa N, Nagamatsu M, Harada S (1994): Molecular cloning of a novel protein tyrosine phosphatase containing a membrane-binding domain and GLGF repeats. FEBS Lett 337:200–206.

Marfatia SM, Lue RA, Branton D, Chishti AH (1994): In vitro binding studies suggest a membrane-associated complex between erytrhoid p55, protein 4.1, and glycophorin C. J Biol Chem 269:8631–8634.

Mauro LJ, Dixon JE (1994): "Zip codes" direct intracellular protein tyrosine phosphatases to the correct cellular "address." Trends Biochem Sci 19:151–155.

Pearson WR, Lipman DJ (1988): Improved tools for biological sequence comparison. Proc Natl Acad Sci USA 85:2444–2448.

Prasad R, Gu Y, Nakamura T, Canaani O, Saito H, Huebner K, Gale RP, Nowell PC, Kuriyama K, Miyazaki Y, Croce CM, Canaani E (1993): Cloning of the ALL-1 fusion partner, the AF-6 gene, involved in acute myeloid leukemias with the t(6;11) chromosome translocation. Cancer Res 53:5624–5628.

Rechsteiner M, Rogers S, Rote K (1987): Protein structure and intracellular stability. Trends Biochem Sci 12:390–394.

Rouleau GA, Merel P, Lutchman M, Sanson M, Zucman J, Marineau C, Hoang-Xuan K, Demczuk S, Desmaze C, Plougastel B, Pulst SM, Lenoir G, Bijlsma E, Fashold R, Dumanski J, de Jong P, Parry D, Eldrige R, Aurias A, Delattre O, Thomas G (1993): Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2. Nature 363:515–521.

Rubinfeld B, Souza B, Albert I, Müller O, Chamberlain SH, Masiarz FR, Munemitsu S, Polakis P (1993): Association of the APC gene product with β-catenin. Science 262:1731–1734.

Saras J, Claesson-Welsh L, Heldin C-H, Gonez LJ (1994): Cloning and characterization of PTPL1, a protein tyrosine phosphatase with similarities to cytoskeletal-associated proteins. J Biol Chem 269:24082–24089.

Sharma S, Birchmeier C, Nikawa J, O'Neill K, Rodgers L, Wigler W (1989): Characterization of the ros1-gene products expressed in human glioblastoma cell lines. Oncogene Res 5:91–100.

Smith DB, Johnson KS (1988): Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. Gene 67:31–40.

Su L-K, Vogelstein B, Kinzler KW (1993): Association of the APC tumor suppressor protein with catenins. Science 262:1734–1737.

Theisen H, Purcell J, Bennett M, Kansagara D, Syed A, Marsh JL (1994): Dishevelled is required during wingless signaling to establish both cell polarity and cell identity. Development 120:347–360.

Trofatter JA, MacCollin MM, Rutter JL, Murrell JR, Duyao MP, Parry DM, Eldridge R, Kley N, Menon AG, Pulaski K, Haase VH, Ambrose CM, Munroe D, Bove C, Haines JL, Martuza RL, MacDonald ME, Seizinger BR, Short MP, Buckler AJ, Gusella JF (1993): A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. Cell 72:791–800.

Tsukita S, Itoh M, Nagafuchi A, Yonemura S, Tsukita S (1993): Submembranous junctional plaque proteins include potential tumor suppressor molecules. J Cell Biol 123:1049–1053.

van Roy F, Mareel M (1992): Tumour invasion: Effects of cell adhesion and motility. Trends Cell Biol 2:163–169.

Walden PD, Cowan NJ (1993): A novel 205-kilodalton testis-specific serine/threonine protein kinase associated with microtubules of the spermatid manchette. Mol Cell Biol 13:7625–7635.

Walton KM, Dixon JE (1993): Protein tyrosine phosphatases. Annu Rev Biochem 62:101–120.

Woods DF, Bryant PJ (1991): The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions. Cell 66:451–464.

Woods DF, Bryant PJ (1993): ZO-1, DlgA and PSD-95/SAP90: Homologous proteins in tight, septate and synaptic cell junctions. Mech Dev 44:85–89.

Yang Q, Tonks NK (1991): Isolation of a cDNA clone encoding a human protein-tyrosine phosphatase with homology to the cytoskeletal-associated proteins band 4.1, ezrin, and talin. Proc Natl Acad Sci USA 88:5949–5953.