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Protein Tyrosine Phosphatase Containing SH2 Domains: Characterization, Preferential Expression in Hematopoietic Cells, and Localization to Human Chromosome 12p12-p13

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Protein tyrosine phosphorylation has been implicated in the growth and functional responses of hematopoietic cells. Recently, approaches have been developed to characterize the protein tyrosine phosphatases that may contribute to regulation of protein tyrosine phosphorylation. One novel protein tyrosine phosphatase was expressed predominantly in hematopoietic cells. Hematopoietic cell phosphatase encodes a 68-kDa protein that contains a single phosphatase conserved domain. Unlike other known protein tyrosine phosphatases, hematopoietic cell phosphatase contains two *src* homology 2 domains. We also cloned the human homolog, which has 95% amino acid sequence identity. Both the murine and human gene products have tyrosine-specific phosphatase activity, and both are expressed predominantly in hematopoietic cells. Importantly, the human gene maps to chromosome 12 region p12-p13. This region is associated with rearrangements in approximately 10% of cases of acute lymphocytic leukemia in children.

The growth and many functional responses of hematopoietic cells are regulated through phosphorylation of proteins on tyrosine. However, most hematopoietic growth factor receptors and antigen receptors do not contain intrinsic protein kinase activity but nevertheless rapidly induce protein tyrosine phosphorylation (22–24, 31, 40, 42, 48). A variety of observations indicate that these phosphorylations are essential for a mitogenic response. In particular, introduction of protein tyrosine kinases has been shown to abrogate the requirements of hematopoietic cells for their growth factors (8, 45) and temperature-sensitive mutants of the v-*abl* kinase confer a temperature-sensitive phenotype for growth factor dependence (7, 27). More recently, it has been shown that there is a direct correlation between the abilities to support growth and initiate protein tyrosine phosphorylation in a series of altered receptors for erythropoietin (41).

The mechanisms by which hematopoietic receptors without intrinsic kinase activity alter protein tyrosine phosphorylation could involve coupling of the receptors to a tyrosine-specific kinase and/or protein tyrosine phosphatase. A role for *src*-related protein tyrosine kinases has been suggested in several studies. In T cells, *lck* associates with CD4 and CD8 and is activated by triggering through these surface receptors (59, 60, 78, 80, 81) and *fyn* associates with the T-cell receptor (56). In mast cells and B cells, *lyn* kinase has been shown to associate with the immunoglobulin E receptor (13) or surface immunoglobulin M (83), respectively. Recent studies have shown that *lck* kinase activity is increased following stimulation of T cells with interleukin 2 (IL-2) (21) and that *lck* associates with the IL-2 receptor β chain (20).

Recent studies have also demonstrated a potential role for protein tyrosine phosphatases in T-cell activation and growth. In particular, T cells which lack the membrane-associated protein tyrosine phosphatase CD45 are unable to respond through the T-cell receptor in either proliferation (46) or protein tyrosine phosphorylation (30). It has been hypothesized that CD45 is required for activation of *src*-related kinase *lck* or *fyn* by dephosphorylation of a carboxyl, regulatory site of tyrosine phosphorylation (28). A role for protein tyrosine phosphatases in hematopoietic cell growth has also been speculated about on the basis of the observation that inhibitors of protein tyrosine phosphatases can substitute for growth factors and induce mitogenic responses (73).

While a variety of hematopoietic cell protein tyrosine kinases have been characterized, less is known about protein tyrosine phosphatases. The known mammalian protein tyrosine phosphatases fall into two broad categories (15). One class is cytoplasmic and includes protein tyrosine phosphatase 1B (PTP1B) (2, 6, 19), a phosphatase that was initially identified in T cells and termed T-cell protein tyrosine phosphatase (TCPTP) (9), neuronal phosphatase (STEP) (34), and cytoplasmic phosphatases that contain a region of homology to cytoskeletal proteins (17, 84). The second class includes the receptorlike phosphatases human tyrosine phosphatase β (HPTPβ) (33), CD45/LCA/Ly5 (72, 74, 76), a leukocyte common antigen-related phosphatase (LAR) (66), human protein tyrosine phosphatase α (HPTPα) (33, 36, 58), human protein tyrosine phosphatase ε (HPTPε) (33), human protein tyrosine phosphatase γ (HPTPγ) (25, 33), and human protein tyrosine phosphatase ζ (HPTPζ) (25, 33).

In studies to identify the protein tyrosine phosphatases in murine IL-3-dependent myeloid cells, three novel phosphatases were identified by polymerase chain reaction (PCR) amplification of cDNAs with oligonucleotide primers to the conserved regions of the known protein tyrosine phosphatases (86). One of these novel phosphatases (PTPty42) was of particular interest because it was expressed predominantly in hematopoietic cells. As demonstrated here, cloning and sequencing of full-length murine and human cDNAs demonstrated that it is a unique, potentially cytoplasmic, 68-kDa protein tyrosine phosphatase which contains a single conserved phosphatase domain in the carboxyl terminus.

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Strikingly, the amino-terminal region of the gene contains two *src* homology 2 (SH2) domains. The human gene is highly homologous to the murine gene. Both the human and murine genes are expressed predominately in hematopoietic cells, and we have therefore designated the gene hematopoietic cell phosphatase (HCP). The levels of the transcripts were not altered by growth factors in the cells examined. Importantly, we demonstrated that human HCP is located on the short arm of chromosome 12, at p11-p13, a region that is frequently involved in translocations or deletions in acute lymphocytic leukemias (ALL).

MATERIALS AND METHODS

Cells and cell culture. All of the cell lines used for these studies were obtained from the American Type Culture Collection, with the exception of those noted. The cells were maintained in RPMI 1640 containing 10% fetal calf serum. The origin and properties of the Mo-7e cell line have been previously described (1). These cells were maintained in RPMI 1640 containing 10% fetal calf serum and 20 U of recombinant human IL-3 per ml. Recombinant human IL-3 was obtained from R&D Systems (Minneapolis, Minn.). The 32Dcl murine myeloid cell line (39) was maintained in RPMI 1640 in the presence of 10% fetal calf serum and murine IL-3 (20 U/ml) purified from conditioned medium of COS cells transfected with a eucaryotic expression vector containing murine IL-3 cDNA.

Cloning of cDNAs for murine and human HCPs. PCR amplification of cDNA was used to obtain portions of potential protein tyrosine phosphatases with oligonucleotide primers to conserved sequences of the known protein tyrosine phosphatases (86). One of these amplified cDNA clones, PTPty42, was used for cloning of cDNAs. To isolate cDNA clones, the 0.38-kb cDNA fragment of PTPty42 (86) was ^{32}P labeled by random priming (Stratagene) and used to screen a cDNA bacteriophage library of mouse bone marrow-derived monocytic cells (87). The longest cDNA clone (2.2 kb) was subcloned into a Bluescript plasmid vector, and the nucleotide sequence was determined in both directions by the chain termination method (57). Human cDNAs were isolated from the Jurkat cell line of human T-lymphoid cells (Stratagene). cDNA inserts from positive phage clones were isolated and subcloned into pBluescript vectors (Stratagene). The longest cDNA insert (2.1 kb) was fully sequenced in both directions by chain termination (57) as previously described (85).

RNA isolation and Northern (RNA) hybridization. Total cellular RNA and poly(A)$^+$ RNA were isolated from cells by procedures that have been previously described (87). Approximately 20 μg of total cellular RNA or 4 μg of poly(A)$^+$ RNA of each sample were separated in 1.2% agarose–formaldehyde gels by electrophoresis and blotted to nitrocellulose filters. The filters were hybridized with ^{32}P-labeled, randomly primed murine or human cDNA fragments and detected by autoradiography. The filters were stripped and rehybridized with a c-*myc* probe (12) or a β-actin probe to assess the levels of RNA.

Immunological methods. Antiserum against murine HCP fused to a T7 gene 10 protein was prepared (69). Briefly, a 700-bp *Apa*I-to-*Eco*RI restriction fragment containing the carboxyl-terminal region of the protein was ligated into the pMEX1 vector (Promega). The fusion protein was expressed in *Escherichia coli*, purified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (64), and used to immunize rabbits by standard procedures. Immunoprecipitations were performed as previously described (85).

Production of HCP and protein tyrosine phosphatase assays. Murine HCP cDNA was restriction digested with *Apa*I and *Eco*RI to release the 700-bp fragment that encodes the C-terminal region of the HCP protein. This fragment was then ligated into the comparable sites in the pMEX1 vector (Promega) to produce a T7 gene 10/HCP fusion protein in *E. coli* (69). The fusion protein was isolated by preparative SDS-PAGE (64) and used to immunize rabbits for anti-HCP serum by standard procedures. Immunoprecipitation, in vitro transcription, and translation of HCP and ^{35}S labeling of 32Dcl cells were performed as described previously (86). Two oligonucleotides (CGAATTCCAGTGAGAACCCC and CGAATTCCCAGATCACTTCCTC) were used as primers to amplify HCP cDNA as follows: 20 cycles of 92°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The PCR product was restriction digested with *Eco*RI and cloned into the *Eco*RI site of the glutathione-S-transferase gene in the pGEX3X vector (Promega). GST/HCP protein was produced and purified by standard procedures (64). Approximately 1 μg of purified GST or GST/HCP protein was separated on SDS–10% PAGE gels and stained with Coomassie blue. The purified proteins were stored at −70°C in 40% glycerol–PTPase buffer (25 mM imidazole [pH 7.4], 0.1% β-mercaptoethanol, 1 mg of bovine serum albumin per ml). Histone VII (Sigma) was ^{32}P labeled with protein kinase A (Sigma) at 30°C for 20 min in buffer A [50 mM sodium acetate (pH 6.5), 20 mM $MgSO_4$, 20 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 20 mM dithiothreitol, histone VII at 2 mg/ml]. Enolase (Sigma) was labeled with *lyn* kinase from M07 cells by previously described procedures (11). These labeled proteins were precipitated with 20% cold trichloroacetic acid, washed three times in 20% trichloroacetic acid, and dissolved in H_2O. Phosphatase assay was performed as described previously (75).

Oligonucleotides to the 5′ and 3′ regions were used as primers to amplify human HCP cDNA as follows: 20 cycles of 92°C for 1 min, 50°C for 1 min, and 72°C for 1 min. The PCR product was restriction digested with *Eco*RI and cloned into the *Eco*RI site of the glutathione-S-transferase gene in the pGEX3X vector (Promega). GST/HCP protein was produced and purified by standard procedures (64). The purified proteins were stored at −70°C in 40% glycerol–PTPase buffer. Histone VII (Sigma) was ^{32}P labeled with protein kinase A (Sigma) at 30°C for 20 min in buffer A. Raytide (Oncogene Sciences) was labeled with v-*abl* kinase and purified as previously described (18). Phosphatase assay was performed as described previously (18).

Isolation of human HCP genomic clones and chromosomal mapping. The 2.1-kb human cDNA was labeled with ^{32}P by random priming and used to screen a lambda dash genomic library from human T lymphocytes (Stratagene). Two clones of 18 and 25 kb were obtained which contained the 3′ region of the human HCP gene. Bromodeoxyuridine-synchronized, phytohemagglutinin-stimulated peripheral blood lymphocytes from a normal donor were used as a source of metaphase chromosomes (88). Purified phage DNA was nick translated with digoxigenin-11 UTP and rehybridized with a chromosome 12-specific biotinylated probe (D12Z1; Oncor, Gaithersburg, Md.) in a hybridization buffer consisting of 65% formamide, 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and 75 μg of Cot 1 DNA (Bethesda Research Laboratories, Gaithersburg, Md.) per ml at 37°C overnight. Hybridization was followed by one 20-min wash

in 50% formamide–2× SSC at 41°C and two 4-min washes in 2× SSC at 41°C. The hybridization signal was detected with fluorescein-conjugated sheep antidigoxigenin antibodies (Boehringer Mannheim, Indianapolis, Ind.) and fluorescein-conjugated avidin (Oncor) as previously described (47). Slides were counterstained in a solution containing 1 μg of propidium iodide per ml, 90% glycerol, and 2% 1,4-diazobicyclo[2.22]octane (Sigma Chemical, St. Louis, Mo.) and examined by fluorography.

Nucleotide sequence accession numbers. The human and murine HCP sequences discussed here have been deposited in the GenBank data base under accession no. M74903 and M68902, respectively.

RESULTS

Molecular cloning and sequencing of HCP cDNAs. To clone the protein tyrosine phosphatases that are expressed in murine IL-3-dependent myeloid cells, we used an approach based on sequence homology and PCR amplification (86). Sequencing of 100 amplified cDNA clones identified seven different cDNA sequences. Two were identical to the murine protein tyrosine phosphatase genes CD45/LCA/Ly5 and HPTPα, and two were highly homologous to human HPTPε and PTP1B. Three encoded novel putative protein tyrosine phosphatases. The amplified cDNA clone for one of these novel phosphatases (PTPty42) detected a 2.6-kb transcript in hematopoietic cells and was used to obtain cDNA clones from a murine monocyte cDNA library (87), and the sequences of the cDNA clones were determined (Fig. 1). Because of the predominant expression of the gene in hematopoietic cells, the term HCP was chosen.

The longest murine cDNA clone was 2,163 bp long and contained a single long open reading frame (ORF) of 1,785 bp that was preceded by a 5′ noncoding region with multiple stop codons. The ATG at the beginning of the ORF was flanked by a sequence that conforms to the Kozak consensus sequence for translation initiation (32). The 3′ noncoding region contains a typical polyadenylation signal motif (ATAAA) followed by a poly(A) tail (16). The ORF encodes a protein of approximately 68 kDa. The carboxyl region (amino acids [aa] 266 to 520) of the predicted protein sequence contains the conserved sequence motifs found in all protein tyrosine phosphatases (Fig. 2A), including a cysteine residue and its flanking sequences (VHC-453 SAGIGRT) that are essential for phosphatase catalytic activity (67).

Carboxyl to the catalytic domain is an 80-aa tail. This region contains a high proportion of charged and polar amino acid residues. This is reminiscent of the carboxyl tail of TCPTP, which inhibits enzyme activity (9). Interestingly, this region also contains three nuclear localizationlike motifs (TTKKKLE, aa 518 to 524; KVKKQRSAD, aa 576 to 584; and KNKGSLKRK, aa 587 to 595) (54). There are three potential sites for N glycosylation.

In reviewing the predicted protein sequence, it was noted that the amino-terminal region of the protein contained two tandem repeats (aa 4 to 84 and 102 to 100) of the sequence motifs that characterize SH2 domains (29). In particular, HCP contains the invariant residues found in SH2 domains (Fig. 2B). Of the three conserved residues that have been postulated to participate in interactions with phosphotyrosine, two are present in HCP.

To isolate cDNAs for human HCP, the murine HCP cDNA was used to screen a cDNA library from a human T-lymphoid cell line. This resulted in isolation of a 2.1-kb cDNA clone that contained a single long ORF of 1,785 bp (data not shown). An ATG is located at the beginning of the ORF and is flanked by sequence motifs commonly found at sites of initiation of translation (32). The presence of multiple stop codons 5′ of the ATG also indicates that this ATP is the most likely translation start site. The ORF of the human cDNA shows 95% amino acid sequence identity (Fig. 1) and 94% nucleotide sequence identity to the murine gene. Of the 32 amino acid differences between the human and murine HCPs, only 5 are nonconservative changes. Thus, the two proteins have 99% conserved amino acid identity. In contrast to the coding region, the nucleotide sequences between the murine and human cDNAs are only approximately 30% similar in the 5′ and 3′ noncoding regions (Fig. 1B).

HCP cDNAs encode an active protein tyrosine phosphatase of 68 kDa. To characterize the protein, polyclonal antibodies against an HCP/T7 phage gene 10 fusion protein were prepared. These antibodies specifically immunoprecipitated a 68-kDa protein from an in vitro translation reaction that utilized in vitro-transcribed RNA from HCP cDNA (Fig. 3A). The antiserum also specifically immunoprecipitated a 68-kDa protein from extracts of a murine myeloid cell line (32Dcl) (Fig. 3A) but not from extracts of NIH 3T3 fibroblasts (data not shown).

To assess the enzymatic activity of HCP, glutathione-S-transferase/HCP fusion proteins were expressed in *E. coli* and purified. As shown in Fig. 3, the murine fusion protein dephosphorylated tyrosine-phosphorylated enolase or raytide (data not shown) but had no activity against serine/threonine-phosphorylated histone VII. Comparable results were obtained with the human HCP fusion protein. In contrast, GST protein alone had no phosphatase activity against either substrate (data not shown). Phosphatase activity against tyrosine-phosphorylated raytide was also detected in immunoprecipitates of HCP from 32Dcl cells (data not shown). The level of this activity was unaffected by the presence or absence of IL-3. The effects of sodium orthovanadate, a potent inhibitor of protein tyrosine phosphatase activity, were also examined. The phosphatase activities of both the murine (Fig. 3C) and human (data not shown) enzymes were inhibited.

Preferential expression of HCP transcripts in hematopoietic cells. Initial studies with probes for the amplified phosphatase domain of murine HCP indicated that it was expressed in hematopoietic cells (86). To examine the pattern of expression further, full-length cDNA clones were used in Northern hybridizations with a variety of tissues and cell lines (Fig. 4). In all cases, a major transcript of approximately 2.6 kb was detected. Among a variety of murine tissues, HCP transcripts were detected primarily in thymus tissue, whole bone marrow, and Ficoll-Hypaque-separated peripheral leukocytes. Low levels of transcripts, possibly due to contaminating blood cells, were observed in kidney, liver, lung, spleen, and other tissues. This pattern of expression is consistent with our previous studies with the phosphatase domain probe and further suggests that HCP is expressed in hematopoietic cells, particularly in thymocytes and bone marrow cells.

Among a series of cell lines (Fig. 5), HCP transcripts were not detected in fibroblasts, nor were they present in a series of epithelial and neuroblastoma cell lines (data not shown). However, transcripts were readily detected in all of the hematopoietic cell lines examined. High levels of transcripts were detected in either a macrophage cell line (Bac1-2F5) or normal bone marrow-derived macrophages. The two B-cell lines examined (plasmacytoma and NFS112) contained low

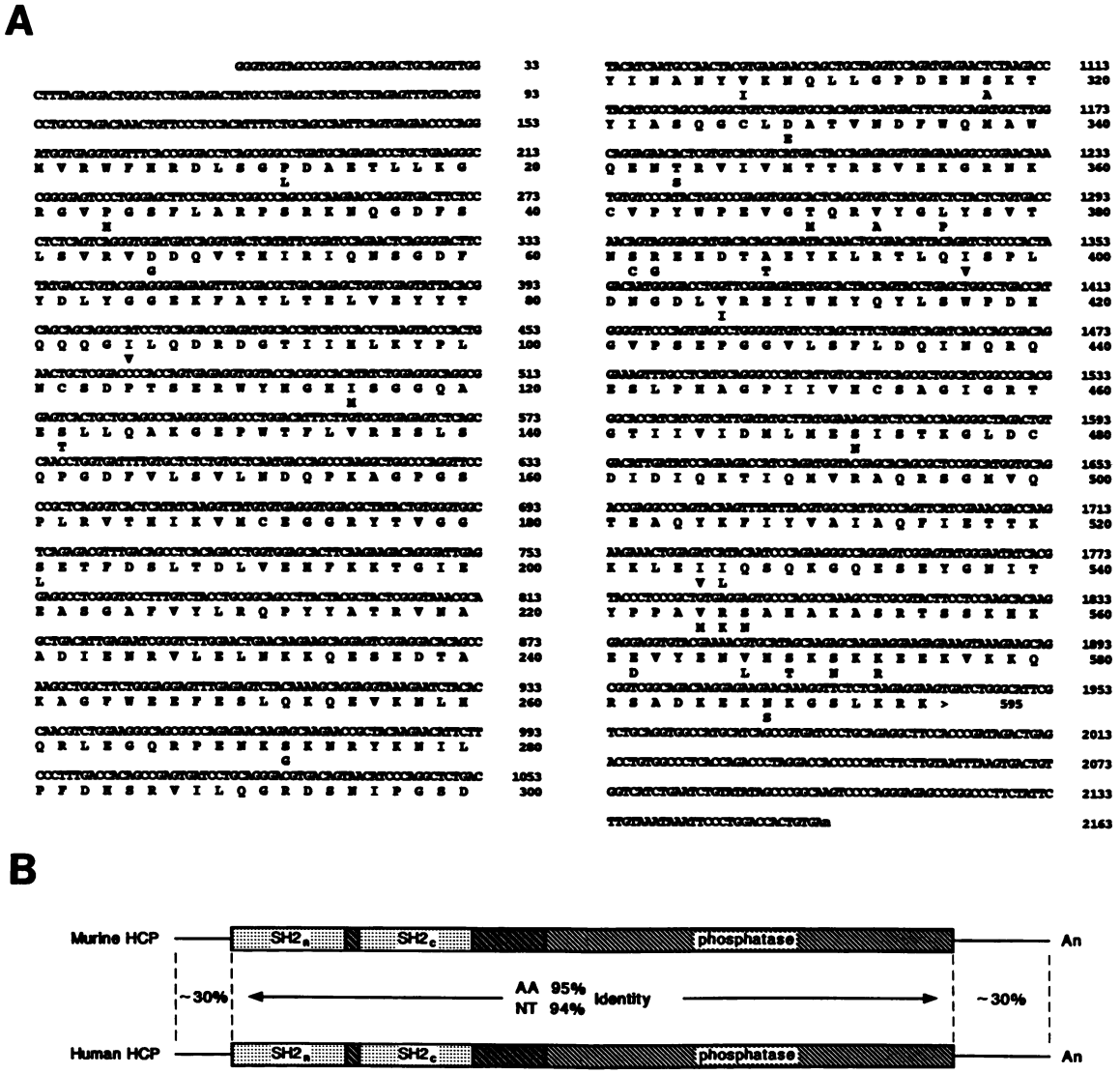

FIG. 1. (A) Nucleotide sequence of a murine HCP cDNA clone. The nucleotide sequence and the predicted amino acid sequence for the ORF region are shown. The amino acids that differ between murine HCP and human HCP are shown beneath the murine HCP protein sequence. (B) Comparison of the structures of the murine and human HCP genes. The ORF region is boxed. The positions of the SH2 domains and the potential protein tyrosine phosphatase catalytic domain are indicated. Within the ORF, there is 95% amino acid (AA) sequence identity and 94% nucleotide (NT) sequence identity, as indicated. The 5′ and 3′ noncoding regions are shown as lines. There is approximately 30% nucleotide sequence similarity in these regions, as indicated.

levels of transcripts. The levels of HCP transcripts were variable among a group of T-cell lines (RL12, EL4, and DA2) and among a series of IL-3-dependent myeloid cell lines (FDC-P1, DA3, NFS58, NFS60, NFS78, DA8, and NFS56). HCP transcripts were also present in the murine IL-3-dependent 32Dcl myeloid cell line.

The levels of transcripts for a number of genes expressed in hematopoietic cells are regulated by growth factors. It was therefore of interest to determine the effects of growth factors on the levels of HCP transcripts. However, neither removal of IL-3 from nor addition of IL-3 to 32Dcl cells had a marked effect on the levels of HCP transcripts (data not shown).

Human cDNA was used a probe in Northern blot hybridizations to assess the levels of expression in various human cell lines (Fig. 6). A transcript of approximately 2.6 kb was detected in all of the hematopoietic cells examined, including monocytic cells (U937 and RWLeu4), myeloid cells (HL-60), a megakaryocytic leukemia cell line (Mo-7e), T-lymphoid cells (MOLT-3, A3.01, and CCRF-CEM), and a B-lymphoid cell line (Raji). Low levels of transcripts were detected in a breast cancer epithelial cell line (MCF7) and a lung carcinoma cell line (A549). However, no HCP transcripts were detected in a human fibroblast cell line (Hs27), a colon epithelial cell line (Colo320), or lung epithelial cell lines (SCLC24 and SCLC22).

Chromosomal mapping of the human HCP gene. Genomic clones containing the human HCP gene were isolated by screening a genomic library with human HCP cDNA. Overlapping genomic clones of 18 (λ4) and 25 (λ8) kb were obtained, and a restriction map was developed (data not shown). To establish that the clones contained the HCP

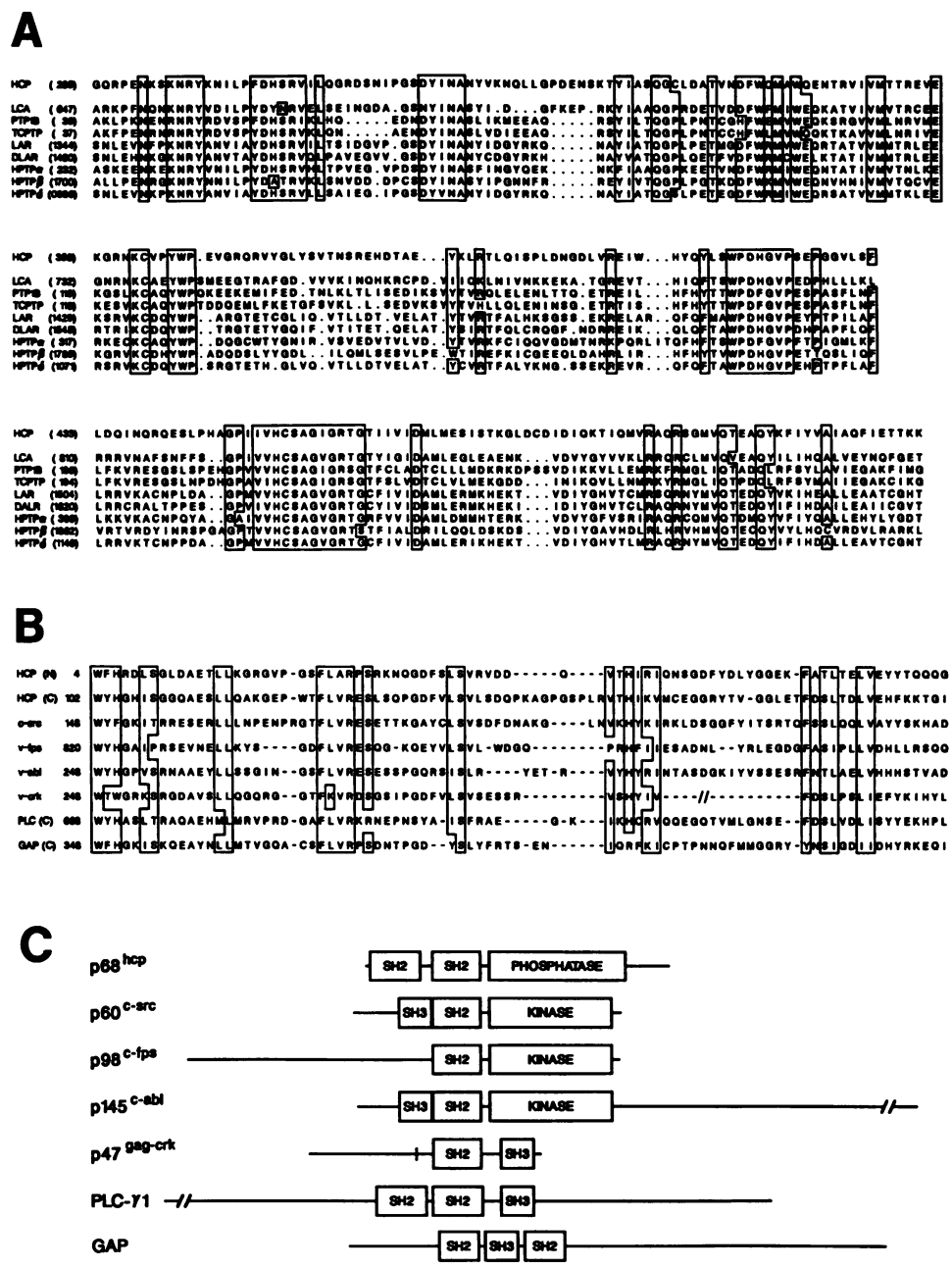

FIG. 2. Sequence alignment of HCP. (A) The catalytic domain sequences of the following protein tyrosine phosphatases were aligned: HCP; LCA (51); PTP1B (4); TCPTP (10); LAR (66); DLAR (68); and HPTPα, HPTPβ, and HPTPγ (33). For those protein tyrosine phosphatases with two catalytic domain repeats, only the N-terminal domain was used. Numbers indicate the first residue shown. The conserved sequences are boxed. (B) Conserved sequences in SH2 domains. The SH2 domain sequences of the following gene products were aligned by eye: HCP(N)/HCP(C), the N- and C-terminal SH2 repeats of HCP; c-*src* (71); v-*fps*/*fes* (62); v-*abl* (52); v-*crk* (38); the phospholipase C-γ (PLC) C-terminal repeat (82); and the GTPase-activating protein (GAP) C-terminal repeat (82). Numbers indicate the first residue shown. The conserved sequences are boxed. (C) Schematic representation of proteins containing an SH2 domain(s). Sequence motifs of SH2, SH3, and the catalytic domains are boxed. Polypeptide sequences unique to each protein are indicated by lines.

gene, the 5′ portion of a 6-kb *Eco*RI-*Bam*HI restriction fragment that hybridized with the cDNA probes was sequenced. This fragment was found to contain two coding exons for the gene which contained sequences 1158 to 1289 and 1757 to 1877 of the cDNA clone. To map the human gene, both genomic clones were used in fluorescence in situ hybridization (47). As illustrated in Fig. 7A, HCP probe λ4 colocalized on chromosome 12 with a chromosome 12-specific centromeric probe. HCP probe λ8 hybridized to the same region (data not shown). HCP probe binding was primarily in the short arm of the chromosome in region p12-p13. No fluorescence signal was seen on any other chromosomes. The position of the hybridization signal ranged from 66 to 90%, with a median value of 75%, of the distance from the centromere to 12 pter, and thus HCP was assigned to bands p12-p13 (Fig. 7B).

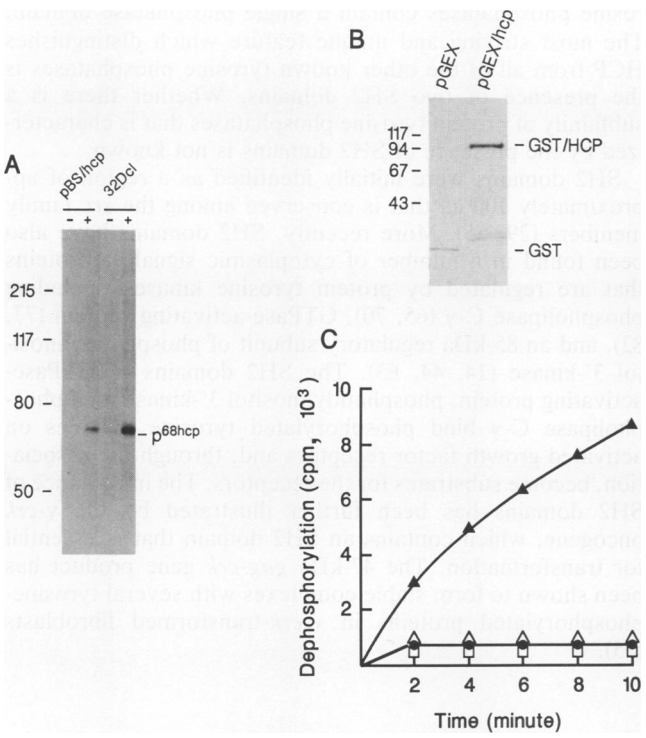

FIG. 3. Detection of HCP protein and protein tyrosine phosphatase activity. (A) Rabbit anti-HCP serum (lanes +) immunoprecipitated a protein of approximately 68 kDa from in vitro translation of HCP RNA (pBS/hcp) and from murine myeloid cells (32Dcl). No protein was detected under similar conditions with preimmunization rabbit serum (lanes −). Molecular size markers (in kilodaltons) are shown on the side. (B) HCP fusion protein GST/HCP was produced in *E. coli*, purified by affinity chromatography, and examined by SDS-PAGE. (C) HCP fusion protein dephosphorylated tyrosyl phosphoenolase (▲) but not serine phosphohistone VII (△). Dephosphorylation of tyrosyl phosphoprotein by HCP fusion protein was inhibited by 1 mM $NaVO_4$ (●). No phosphatase activity was detected for GST protein (□). In these assays, 10^3 cpm corresponds to approximately 0.5 nmol of P_i. The initial reaction rate is, therefore, approximately 0.7 nmol/min.

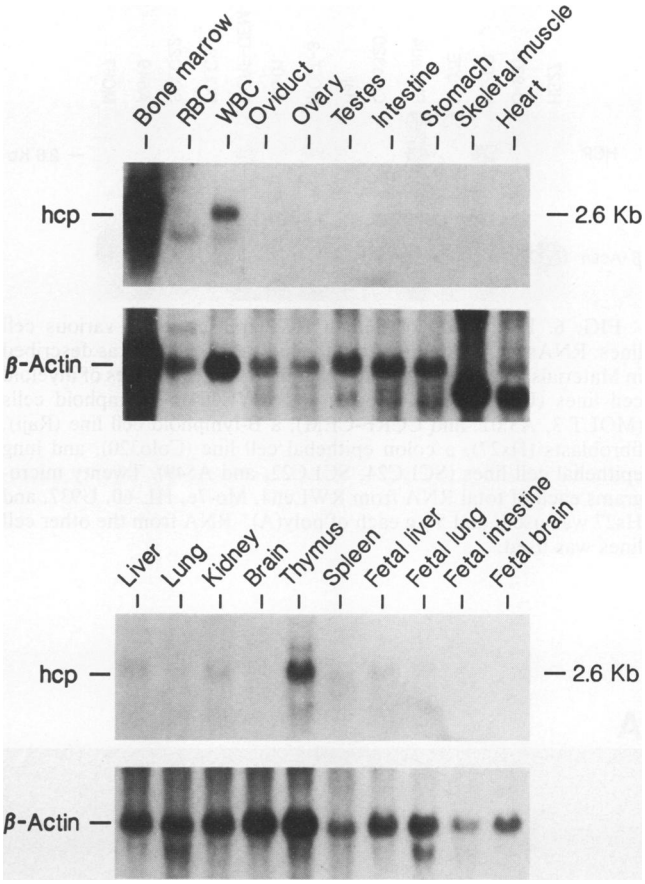

FIG. 4. Expression of HCP in murine tissues. HCP expression was examined by Northern hybridization using the 2.2-kb murine HCP cDNA as the probe (86). Approximately 20 μg of total cellular RNA or 2 μg of poly(A)$^+$ RNA isolated from different mouse tissues and organs was used for hybridization. A β-actin probe was used to examine the same blots, and the results are shown in the lower panels. The size of the HCP transcript and the position of the β-actin probe are marked on the sides.

DISCUSSION

Since the initial purification, sequencing, and cloning of a protein tyrosine phosphatase (2, 4–6, 19), additional potential protein tyrosine phosphatases have been identified at a rapid pace. This has largely been due to the identification of conserved sequences among the protein tyrosine phosphatases and the use of PCR approaches to amplify novel genes from cDNA. This approach has allowed identification of novel protein tyrosine kinases in cDNAs from sources as diverse as mammalian tissues (17, 43, 84) and sea squirts (37). Our efforts have focused on the protein tyrosine phosphatases of IL-3-dependent myeloid cells and initially resulted in the identification of conserved domains for three potentially novel protein tyrosine phosphatases in these cells (86). One of these (PTPty42) was of particular interest because it was expressed predominantly in hematopoietic cells.

To date, the protein tyrosine phosphatases have fallen into two broad categories, which are receptorlike and membrane associated or cytosolic. A further distinction can be made on the basis of the presence of one or two phosphatase conserved domains, although all known cytosolic protein ty-

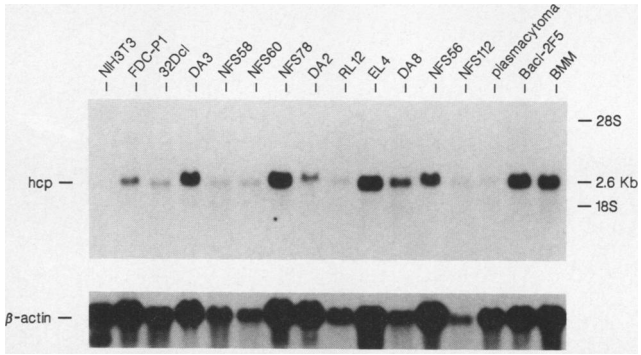

FIG. 5. Expression of HCP in various murine cell lines. Northern hybridization of RNAs from in vitro-cultured (86) fibroblasts (NIH 3T3), myeloid cells (FDC-P1, 32Dcl, DA3, NFS58, NFS60, and NFS78), T-lymphoid cells (DA2, RL12, EL4), B-lymphoid cells (DA8, NFS56, NFS112, and J3 plasmacytoma), and monocytic cells (Bac1-2F5 and mouse bone marrow-derived monocytic cells [BMM]). Hybridizations were done as described in Materials and Methods.

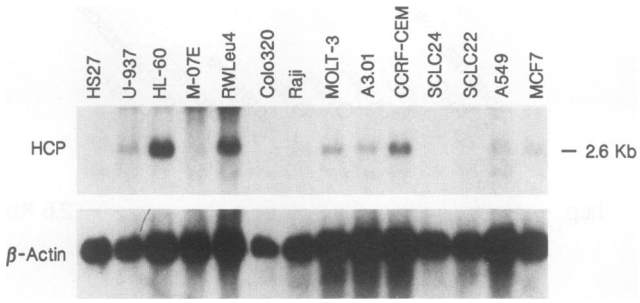

FIG. 6. Expression of human HCP transcripts in various cell lines. RNAs were obtained from the indicated cell lines as described in Materials and Methods. The cell lines included a series of myeloid cell lines (U937, HL60, Mo-7e, and RWLeu4), T-lymphoid cells (MOLT-3, A3.01, and CCRF-CEM), a B-lymphoid cell line (Raji), fibroblasts (Hs27), a colon epithelial cell line (Colo320), and lung epithelial cell lines (SCLC24, SCLC22, and A549). Twenty micrograms each of total RNA from RWLeu4, Mo-7e, HL-60, U937, and Hs27 was used, and 5 μg each of poly(A)$^+$ RNA from the other cell lines was used.

rosine phosphatases contain a single phosphatase domain. The most striking and unique feature which distinguishes HCP from all of the other known tyrosine phosphatases is the presence of two SH2 domains. Whether there is a subfamily of protein tyrosine phosphatases that is characterized by the presence of SH2 domains is not known.

SH2 domains were initially identified as a region of approximately 100 aa that is conserved among the *src* family members (29, 55). More recently, SH2 domains have also been found in a number of cytoplasmic signaling proteins that are regulated by protein tyrosine kinases, including phospholipase C-γ (65, 70), GTPase-activating protein (77, 82), and an 85-kDa regulatory subunit of phosphatidylinositol 3′-kinase (14, 44, 63). The SH2 domains of GTPase-activating protein, phosphatidylinositol 3′-kinase, and phospholipase C-γ bind phosphorylated tyrosine residues on activated growth factor receptors and, through the association, become substrates for the receptors. The importance of SH2 domains has been further illustrated by the v-*crk* oncogene, which contains an SH2 domain that is essential for transformation. The 47-kDa *gag-crk* gene product has been shown to form stable complexes with several tyrosine-phosphorylated proteins in v-*crk*-transformed fibroblasts (35).

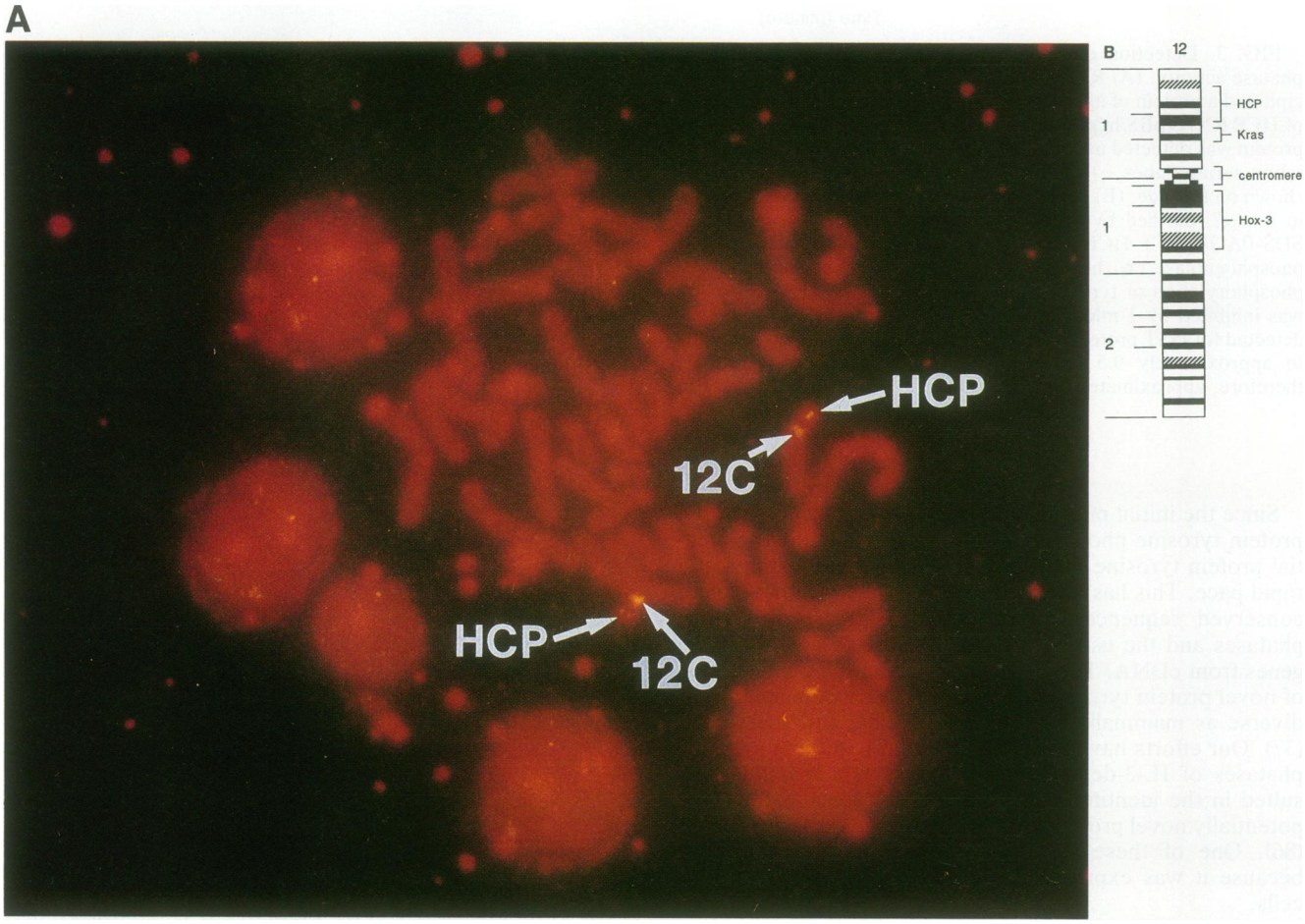

FIG. 7. (A) Localization of the gene for HCP to chromosome 12p13 by fluorescence in situ hybridization. Human HCP genomic clone λ4 was cohybridized with a probe specific for the centromere of chromosome 12 to metaphase spreads of phytohemagglutinin-stimulated human peripheral blood lymphocytes. The HCP probe hybridized to the p12-p13 region on both chromatids of chromosome 12, as indicated by the arrows. The hybridization position of the probe for the chromosome 12 centromeric region is also indicated by arrows (12C). (B) Location of the gene for HCP on chromosome 12 relative to other genes.

The presence of SH2 domains in a protein tyrosine phosphatase suggests that in some cases similar types of protein associations are involved in regulating both phosphorylations and dephosphorylations. The SH2 domains in HCP may be involved in directing its association with a phosphorylated protein tyrosine kinase which, through the association or through phosphorylation, may modify the phosphatase activity of HCP. Alternatively, the SH2 domains may direct the association of HCP with its substrates. In this case, phosphorylation at one site may allow association of a substrate with HCP and result in dephosphorylation at a distant site. For example, recognition of *src* Tyr-416 may promote the association of *src* with HCP and result in dephosphorylation of Tyr-527 with the net consequence of maximal activation of kinase activity. Alternatively, recognition of Tyr-527 and dephosphorylation of Tyr-416 would decrease kinase activity. Experiments are in progress to determine whether the *src*-related kinases in hematopoietic cells are substrates for HCP.

The high level of HCP expression in macrophage cell lines suggested that it associates with the activated colony-stimulating factor 1 receptor. Previous studies (53, 79) have shown that the p85 subunit of phosphatidylinositol 3′-kinase associates with and is phosphorylated by the colony-stimulating factor 1 receptor. This association requires the kinase insert region and has been hypothesized to involve association of the SH2 domains of p85 with the major autophosphorylation site of the colony-stimulating factor 1 receptor. However, we have been unable to detect association of HCP with the activated colony-stimulating factor 1 receptor by immunoprecipitation or Western blotting (immunoblotting) (data not shown). Nor have we been able to identify a protein that consistently coprecipitates with HCP in immunoprecipitates under various conditions of cell stimulation. Irrespective, it will be important to develop additional approaches to identify the proteins that associate with HCP through the SH2 domains and/or substrates for HCP.

The results presented here demonstrate that the human and murine genes show extensive sequence identity. Importantly, all of the critical amino acids in the SH2 domains and in the presumed phosphatase catalytic domain are conserved. The pattern of expression of the gene is also conserved and is found predominantly in hematopoietic cells. The significance of the low levels of transcripts detected in a breast cancer epithelial cell line and one lung carcinoma cell line is not known. Low levels of transcripts for the murine gene were found in liver, lung, and kidney tissues, and thus, there may be some low level of expression in cells of nonhematopoietic lineages.

The predominant expression of HCP in hematopoietic cells suggests that it plays a specific role in the growth or responses of these cells and suggests the possibility that HCP activity is regulated by hematopoietic growth factors. However, there was no effect of IL-3 removal or readdition on the levels of transcripts. 32Dcl cells also have the ability to differentiate terminally to neutrophilic granulocytes when they are cultured in granulocyte-specific colony-stimulating factor. The levels of HCP transcripts were also not markedly modulated during granulocyte-specific colony-stimulating factor-induced terminal differentiation (data not shown). In addition, in preliminary experiments we did not observe an effect of IL-3 on the levels of phosphatase activity in HCP immunoprecipitates.

The human HCP gene was localized to chromosome 12p12-p13. This region is of particular interest because it is a common site of chromosomal rearrangements in ALL and is found in approximately 10% of all cases of childhood ALL (3, 49, 50). These cases of ALL are most commonly early pre-B- or T-cell leukemias. The rearrangements that are most commonly observed involve formation of dicentric chromosomes from a break and loss of the p arms of chromosomes 7 and 12, dic(7;12)(p11;p12). Less commonly involved rearrangements involve dic(12;17)(p11;p12) and t(12;13)(p13;q14). Abnormalities of chromosome 12p13 have also been associated with malignant proliferation of eosinophils (26). Experiments are in progress to assess the role of HCP in cases of ALL with rearrangements of 12p12-p13.

Following completion of our studies, a report (61) appeared which described the cloning of a protein tyrosine phosphatase (PTP1C) containing two SH2 domains from a human breast cancer cell line. Comparison of the sequence of the human HCP cDNA clone with that of PTP1C cDNA indicated that they are virtually identical, with three exceptions. (i) The sequence at amino positions 74 and 75 in HCP is GAG CTG (Glu Leu), while in PTP1C the sequence is GAC GTG (Asp Val). (ii) A sequence difference lies in the carboxyl region of the coding sequence. The human HCP sequence is AGC AAG GGT TCC (Ser-588 Lys Gly Ser), while the reported sequence for PTP1C is AGC AAG GTT CCC (Ser-590 Lys Val Pro). The additional G in the HCP sequence changes the coding frame and results in a change in the terminal 6 aa of HCP and the absence of 12 terminal aa found in PTP1C. The basis for the difference is not known; however, the murine HCP sequence terminates at the same position as the human HCP sequence and the human and murine sequences diverge considerably following the terminator codon. Furthermore, sequencing of the human genomic DNA for this region revealed an exon sequence identical to the HCP cDNA sequence (unpublished data). Therefore, either there is an error in the published PTP1C sequence or a mutation may have occurred in the breast cancer cell line from which the cDNA was isolated. (iii) The third difference is more substantial and is found in the 5′ region. In particular, the 5′ sequence for the noncoding region and the coding region for the first three amino acids of HCP is different from the sequence reported for PTP1C. From sequencing of genomic DNA, this region of the HCP 5′ sequence is contained within an exon which is separated from the next exon by 85 bp (unpublished data). The point of convergence of the HCP and PTP1C sequences corresponds to the latter exon. Therefore, it is possible that the HCP and PTP1C cDNAs utilized different 5′ exons and, possibly, different promoters, which may be related to the different cell lines from which the cDNAs were isolated. This possibility is being examined. Because of the distinctively hematopoietic lineage-specific expression of the gene, we propose that the more descriptive term HCP be used to designate this new phosphatase.

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