Identification of a cytoplasmic, phorbol ester-inducible isoform of protein tyrosine phosphatase ε

(delayed early response/induction/differentiation)

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ABSTRACT   The protein-tyrosine phosphatase ε (PTPε) is a transmembranal, receptor-type protein that possesses two phosphatase catalytic domains characteristic of transmembranal phosphatases. Here we demonstrate the existence of a nontransmembranal isoform of PTPε, PTPε-cytoplasmic. PTPε-cytoplasmic and the transmembranal isoform of PTPε have separate, nonoverlapping expression patterns. Further, the data clearly indicate that control of which of the two isoforms is to be expressed is initiated at the transcriptional level, suggesting that they have distinct physiological roles. PTPε-cytoplasmic mRNA is the product of a delayed early response gene in NIH 3T3 fibroblasts, and its transcription is regulated through a pathway that requires protein kinase C. The human homologue of PTPε-cytoplasmic has also been cloned and is strongly up-regulated in the early stages of phorbol 12-tetradecanoate 13-acetate-induced differentiation of HL-60 cells. Sequence analysis indicates and cellular fractionation experiments confirm that this isoform is a cytoplasmic molecule. PTPε-cytoplasmic is therefore the initial example to our knowledge of a nontransmembranal protein-tyrosine phosphatase that contains two tandem catalytic domains.

Reversible phosphorylation of tyrosine residues in proteins plays a central role in normal cell growth and division. Intracellular levels of phosphotyrosine are controlled by the opposing actions of protein-tyrosine kinases and protein-tyrosine phosphatases (PTPases; reviewed in refs. 1 and 2). PTPases comprise a large and complex family of related molecules that can broadly be divided into two major groups; these are the transmembranal, receptor-like PTPases and the nontransmembranal PTPases (reviewed in refs. 3–5). Transmembranal PTPases contain an extracellular region that is thought to mediate binding of as-yet-unidentified ligand molecules. The intracellular portion of this class of molecules generally contains two PTPase catalytic domains arranged in tandem. All presently known nontransmembranal PTPases contain a single copy of the PTPase domain flanked by protein sequences that regulate the activity or the subcellular localization of the entire molecule.

We have demonstrated that the transmembranal PTPase ε (PTPε) (6) is overexpressed in mouse mammary tumors initiated by v-Ha-*ras* or *neu*, but not by c-*myc* or *int-2* (7). During the course of this work, it became apparent that another major species of PTPε mRNA exists. The expression patterns of the two mRNA species are not similar, indicating that they are not physiologically equivalent. In what follows, we describe the cloning of this variant mRNA and the characterization of its product. The previously unknown isoform, called "PTPε-cytoplasmic," lacks the signal peptide and the extracellular and transmembranal domains of the receptor-type isoform. Cellular fractionation experiments reveal that PTPε-

cytoplasmic is a nontransmembranal molecule, making it to our knowledge the initial member of its class to possess two PTPase domains. In addition, PTPε-cytoplasmic mRNA is a delayed early response gene product in NIH 3T3 fibroblasts, and its transcription there is regulated through a pathway that requires protein kinase C (PKC). PTPε-cytoplasmic is also strongly and rapidly induced in HL-60 cells as they are induced to undergo differentiation by phorbol 12-tetradecanoate 13-acetate (TPA). The expression pattern of this isoform of PTPε suggests that its physiological role may be found in the context of the hematopoietic system.

MATERIALS AND METHODS

Cell Culture. Mammary gland tumors arose in mice carrying the mouse mammary tumor virus/c-*neu* transgene [cell line SMF (8)] or the ζ-globin–v-Ha-*ras* transgene [cell lines AC236 and AC816 (9)]. Adherent cells were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (vol/vol) bovine serum, 2 mM glutamine, and 100 units of penicillin and 100 μg of streptomycin per ml. HL-60 cells were grown in RPMI 1640 medium supplemented with 10% (vol/vol) fetal calf serum and glutamine and antibiotics as above.

For PTPε stimulation experiments, NIH 3T3 cells were incubated in DMEM containing glutamine, antibiotics, and 0.5% bovine serum for 48 hr. Cells were stimulated by the addition of fetal calf serum to 20% final concentration, TPA [Sigma, 100 nM in dimethyl sulfoxide (DMSO) vehicle, 0.1% final concentration], 100 ng of basic fibroblast growth factor (bFGF; Sigma) per ml, 1 μg of epidermal growth factor (EGF, Collaborative Research) per ml, 25 μM forskolin (Sigma) in 1% DMSO vehicle, or 1 mM dibutyryl-cAMP (Sigma), either with or without 10 μg of cycloheximide (CHX; Sigma) per ml. PKC was removed from NIH 3T3 fibroblasts by preincubation with TPA as described (10). To induce differentiation of HL-60 cells, ≈3 × 10⁶ cells were grown for various lengths of time in 0.1% DMSO containing 15 nM TPA; 0.1% DMSO vehicle was determined to have no effect on HL-60 cells.

RNA Analysis and Probes. RNA was isolated by using the guanidinium isothiocyanate/CsCl gradient technique (11) and was purified by oligo(dT) column chromatography (mRNA purification kit, Pharmacia). The murine PTPε probe contained nucleotides 993–1323 from the first catalytic domain of the PTPε cDNA (7). A human PTPε probe, corresponding to nucleotides 652–1408 of the murine PTPε-cytoplasmic cDNA, was generated by PCR on HL-60 cDNA with primers AER2 (GTGTGTAGAGGACTGCGTG) and AEF2 (antisense, CA-TCCTCCAGAAGTCCTCACC). Also used were the c-fos

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Abbreviations: PTPase, protein-tyrosine phosphatase; PTPε, PTPase ε; TPA, phorbol 12-tetradecanoate 13-acetate; DMSO, dimethyl sulfoxide; PKC, protein kinase C; CHX, cycloheximide; RACE, rapid amplification of cDNA ends; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor.
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†The sequences reported in this paper have been deposited in the GenBank data base (accession numbers U36758 and U36623).

cDNA (12), a 320-bp-long *Pvu* II fragment from exon 2 of the murine c-*myc* gene (13), and the rat 28S ribosomal RNA gene (14). A probe for murine *Erp/Mkp1* was generated by PCR amplification of cDNA from NIH 3T3 cells after stimulation with dibutyryl-cAMP (based on data from ref. 15). Oligomers used were Erp4 (TGGGGGGTGAGGGGGGGTGTTAAA) and Erp5 (AGCCACCATCTGCCTTGCTTACC), which amplify bases 1889–2330 of Erp/Mkp1 cDNA (15).

cDNA Library Construction, Screening and 5′ Rapid Amplification of cDNA Ends (RACE). cDNA libraries were constructed in the Lambda Zap vector (Stratagene) with RNA from serum-starved NIH 3T3 cells 2 hr after TPA stimulation. Libraries were screened with the mouse PTPε cDNA probe. 5′ RACE (16) was performed by using the 5′ AmpliFINDER RACE kit (Clontech). The positions of all of the oligomers used are depicted in Fig. 5B. Briefly, RNA was reverse-transcribed by using oligomer E1225 (CTCTGCTGCTCTTG-CTCTTCT). The 3′ end of the first-strand cDNA was ligated to the AmpliFINDER anchor oligomer (Clontech) followed by PCR amplification with this oligomer and oligomer E1225. A similar protocol, using HL-60 mRNA and replacing oligomer E1225 with oligomer HE1225 (CTTTGCTGCTCTTGCTCCT-CC), was used to obtain the human PTPε-cytoplasmic DNA fragment.

Protein Blot Analysis. Cells and organs were homogenized in buffer A (10 mM Tris chloride, pH 7.5/10 mM NaCl/3 mM MgCl$_2$/0.5% Nonidet P-40/0.5 mM phenylmethylsulfonyl fluoride and centrifuged at 4°C for 10 min at 10,000 × g. Supernatants were analyzed on 7.5% polyacrylamide/SDS gels and blotted onto Immobilon-P membranes (Millipore) as described (17). Primary antibody for PTPε detection was rabbit polyclonal anti-PTPε antiserum (7).

Subcellular Fractionation of HL-60 Cells. For isolation of nuclear and nonnuclear fractions, HL-60 cells were washed in phosphate-buffered saline and lysed in buffer A. Lysates were incubated on ice for 10 min, followed by centrifugation at 10,000 × g and 4°C. Both fractions were analyzed by SDS/PAGE and immunoblotting. In separate experiments, cells were incubated on ice for 10 min in hypotonic buffer (1 mM Hepes adjusted with NaOH to pH 7.9/1.5 mM MgCl$_2$/10 mM KCl/0.5 mM dithiothreitol/0.5 mM phenylmethylsulfonyl fluoride) and then lysed by 20 strokes in a Dounce homogenizer. Nuclei and unlysed cells were removed by centrifugation at 10,000 × g, and the supernatant was centrifuged at 100,000 × g and 4°C for 30 min. This last supernatant (S100) contained the cytosolic fraction; the pellet, containing the membranal fraction, was resuspended in 1 ml of hypotonic buffer and centrifuged at 100,000 × g (P100). Both fractions were analyzed by SDS/PAGE and immunoblotting.

RESULTS

Identification of a PTPε Transcript Expressed Preferentially in Hematopoietic Organs. PTPε mRNA is found predominantly in the brain, testes, and lungs, with lower levels present in lymph nodes, thymus, spleen, heart, and mammary glands (Fig. 1 and results not shown). At least four distinct mRNA species of PTPε exist (bands A–D, Fig. 1), all of which originate in the PTPε gene (see below and data not shown). Two of these—marked as bands A and B in Fig. 1—are predominant, and we have previously identified band A as encoding the transmembranal form of PTPε (PTPε-transmembranal; ref. 7). There is little overlap in the expression patterns of these two predominant forms of PTPε mRNA, suggesting that they are physiologically distinct.

The Shorter PTPε mRNA Is a TPA-Inducible, Delayed Early Response Gene Product in NIH 3T3 Fibroblasts. To define some of the physiological differences between the two major PTPε mRNAs, we turned to NIH 3T3 fibroblasts, which express both PTPε mRNAs at low levels (Fig. 2, control lane).

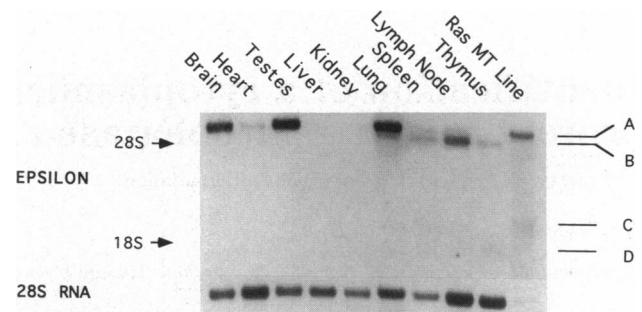FIG. 1.   Expression of PTPε mRNA in various adult mouse organs. Each lane contains 1.5 µg of poly(A)$^+$ RNA. Bands A–D are alternative forms of PTPε mRNA as discussed in the text. Equal loading of RNA was verified by probing with a 28S ribosomal RNA probe. Ras MT line refers to the mammary tumor cell line AC816; note that this lane is severely underloaded.

Stimulation by serum of serum-starved fibroblasts induced the shorter mRNA species (Fig. 2, band B, PTPε-cytoplasmic). Induction was evident 2 hr after addition of serum, peaked at 4 hr, and had significantly receded by 8 hr. No induction of PTPε-transmembranal mRNA was observed in these experiments. fos mRNA, used here as a positive control, was induced as expected 20–40 min after stimulation.

Serum-starved fibroblasts were then challenged with a variety of effectors. TPA was found to induce PTPε-cytoplasmic mRNA with the same time course as serum but to higher levels (Fig. 3 *Upper* and results not shown). EGF and bFGF also induced PTPε-cytoplasmic mRNA, while dibutyryl-cAMP and forskolin did not (Fig. 3 *Upper*). Stimulation in the presence of the protein synthesis inhibitor CHX prevented the induction of PTPε-cytoplasmic mRNA (Fig. 3 *Upper*, lanes 1–4), suggesting that this isoform is the product of a delayed early response gene (18). The induction of PTPε-cytoplasmic then relies on a previous step, probably the synthesis of an unidentified immediate early gene product. All of the effectors used were active, as judged by their ability to induce *Erp/Mkp-1* (Fig. 3 *Upper*). *Mkp-1* is an immediate early gene and is known to be induced by stimulators of pathways involving protein kinase A or PKC (15, 19).

Induction of PTPε-Cytoplasmic mRNA Is Dependent upon PKC Activity. The strongest inducer of PTPε-cytoplasmic mRNA was TPA, a phorbol ester that directly binds and activates PKC (reviewed in refs. 20 and 21). To examine whether PKC plays a role in the induction of PTPε-cytoplasmic mRNA, NIH 3T3 cells were rendered PKC-deficient by pretreatment with TPA (10). When these cells were serum-starved and then re-fed, induction of PTPε-cytoplasmic mRNA was severely reduced
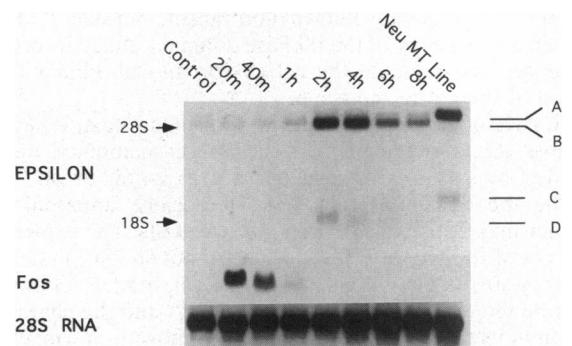

FIG. 2.   Stimulation of quiescent NIH 3T3 cells with 20% serum up-regulates PTPε-cytoplasmic mRNA. The control is unstimulated, serum-starved cells. Neu MT line is the mouse mammary tumor virus/*neu*-initiated mammary tumor cell line SMF. Poly(A)$^+$ RNA (1.5 µg per lane) was loaded and probed with PTPε or 28S ribosomal RNA fragment; fos mRNA induction was examined by using a separate blot containing 10 µg of total RNA per lane. Epsilon, PTPε.

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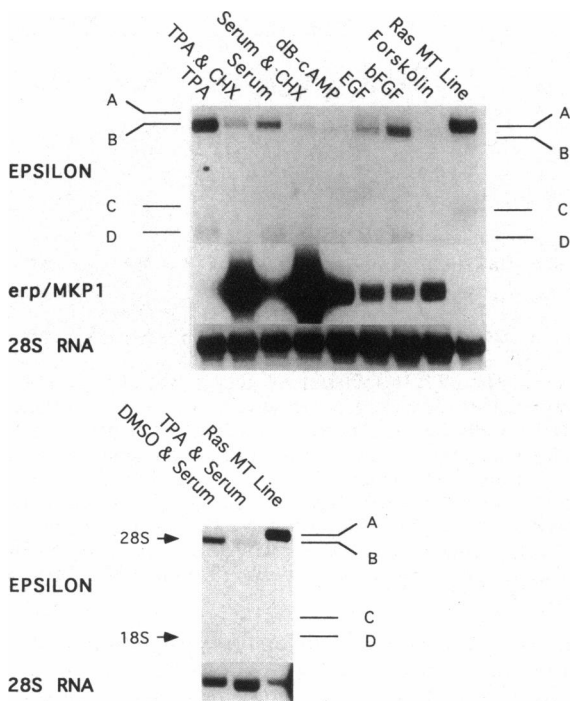
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FIG. 3. (*Upper*) Effect of various treatments on the induction of PTPε mRNA in NIH 3T3 cells. Quiescent cells were stimulated with various effectors, and poly(A)$^+$ RNA was collected 2 hr later. dB-cAMP, Dibutyryl-cAMP; Ras MT line, mammary tumor cell line AC236. The blot was probed for Erp/Mkp1 mRNA as a positive control for the action of the various effectors. (*Lower*) Down-regulation of PKC prevents induction of PTPε mRNA by serum. PKC was down-regulated in NIH 3T3 cells by TPA pretreatment, followed by stimulation with 20% fetal calf serum; poly(A)$^+$ RNA was collected 2 hr later. Control cells (marked "DMSO & serum") were treated with vehicle alone prior to stimulation. Epsilon, PTPε.

(Fig. 3 *Lower*, lane 2). Control cells that had been pretreated with vehicle alone retained their ability to induce PTPε-cytoplasmic mRNA (Fig. 3 *Lower*, lane 1). These results indicate that induction of mRNA of the cytoplasmic isoform in NIH 3T3 cells is dependent upon PKC activity.

The PTPε-Cytoplasmic mRNA Is Rapidly Up-Regulated in the Course of TPA-Induced Differentiation of HL-60 Cells. Treatment of human promyelocytic leukemia HL-60 cells with TPA causes them to differentiate along the monocyte/macrophage pathway (22). As TPA strongly induces PTPε-cytoplasmic mRNA in fibroblasts, we asked whether TPA
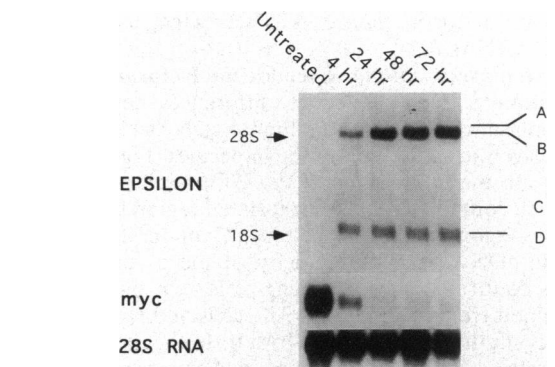

FIG. 4. TPA induces PTPε-cytoplasmic mRNA in HL-60 cells. Poly(A)$^+$ RNA was prepared from HL-60 cells at the indicated times after addition of TPA. The decrease in myc mRNA was used to follow the progress of differentiation.

could induce PTPε here as well. HL-60 cells were treated with TPA for up to 4 days, during which time the cells ceased to divide, became adherent, adopted characteristic morphology (not shown), and down-regulated *myc* expression (Fig. 4; refs. 23 and 24). Analysis of RNA collected throughout this period revealed significant induction of PTPε-cytoplasmic mRNA (Fig. 4, band B). The induced RNA was clearly evident as early as 4 hr after commencement of treatment, well before any changes in the appearance of the cells were noted. Its identity as the human homologue of the murine PTPε-cytoplasmic isoform was confirmed by cDNA cloning and protein blot analysis (see below). A second, smaller PTPε mRNA species was induced along with PTPε-cytoplasmic mRNA (Fig. 4, band D). We believe that this band represents an alternative form of the PTPε-cytoplasmic transcript (A.E., unpublished results).

Cloning and Structure of the PTPε-Cytoplasmic cDNA. To further characterize this inducible form of PTPε, we cloned the murine PTPε-cytoplasmic cDNA from libraries prepared from NIH 3T3 cells 2 hr after TPA stimulation, at the height of PTPε-cytoplasmic mRNA induction. Screening was initiated with a fragment of the murine PTPε-transmembranal cDNA (7). Analysis of a series of overlapping clones revealed that the PTPε-cytoplasmic cDNA is 4961 bp long and includes 1926 bp of coding sequences (Fig. 5A, lower cDNA diagram). A 43-bp-long 5′ untranslated region precedes the initiator ATG, which is conserved in the human homologue of PTPε-cytoplasmic (see below). The 2992-bp 3′ untranslated region, which is identical to that of PTPε-transmembranal, contains
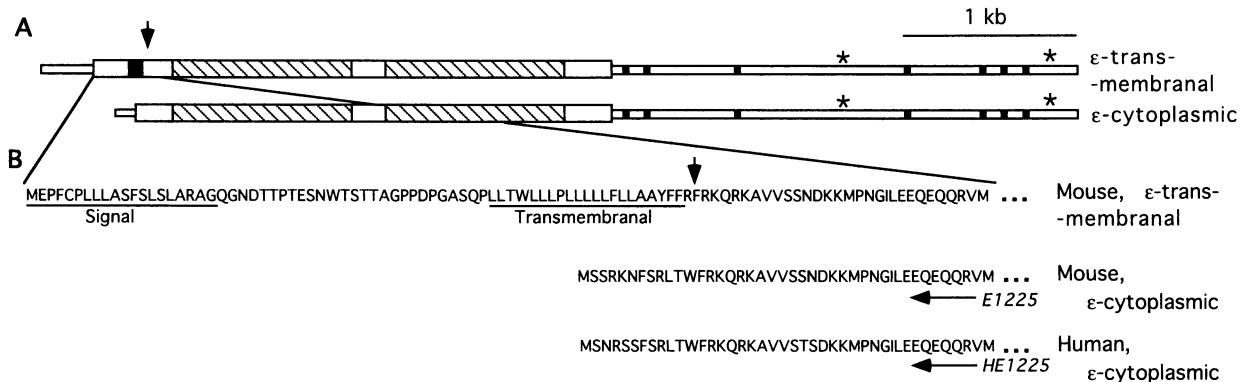

FIG. 5. The PTPε cDNAs and partial protein products. (*A*) Diagram of PTPε-transmembranal and -cytoplasmic cDNAs. Shown are the coding region (thick rectangles) and 5′ and 3′ untranslated regions (thin rectangles) encompassing the transmembranal domain (black box), PTPase catalytic domains (hatched boxes), ATTTA signals (thin black boxes), and polyadenylylation signals (asterisks). Vertical arrows mark where the sequences converge. (*B*) Sequences of amino termini of murine PTPε-transmembranal and -cytoplasmic and human PTPε-cytoplasmic proteins. Horizontal arrows indicate the location and orientation of oligomers used for 5′ RACE and hybridization.

seven repeats of the sequence ATTTA, which is found in short-lived mRNAs (25).

PTPε-Cytoplasmic mRNA Encodes an Isoform of PTPε with a Unique 5′ End. Sequence differences between the PTPε-cytoplasmic and -transmembranal cDNAs (7) were located exclusively at the 5′ ends of the molecules (Fig. 5 and not shown). In all, the first 528 bp of the PTPε-transmembranal cDNA, which contained the 5′ untranslated region and part of the coding region, were replaced by 77 bp in the PTPε-cytoplasmic cDNA. The authenticity of the 5′ end of this cDNA was confirmed by performing 5′ RACE on poly(A)$^+$ RNA obtained from murine NIH 3T3 cells and from human HL-60 cells, both treated with TPA. In both cases, similar sequences were obtained, with the murine sequence identical to the one cloned from the cDNA library described above (Fig. 5B and not shown). The human PTPε-cytoplasmic cDNA contains an in-frame stop codon upstream of the initiator ATG, indicating that it is the true initiating codon (not shown). The nucleotide sequences of the murine PTPε-cytoplasmic and -transmembranal cDNAs were identical downstream of the point where their sequences converged (Fig. 5).

The differences between the PTPε-cytoplasmic and -transmembranal cDNAs extended well into their coding regions. The coding sequences unique to the transmembranal protein product encompassed 69 amino acid residues that included the signal sequence, the extracellular region, and the transmembranal domain. These regions were replaced in the cytoplasmic protein by a stretch of 12 amino acids that lacked these features (Fig. 5B). This finding indicated that the shorter form of PTPε was probably a cytoplasmic molecule, as was subsequently demonstrated.

The PTPε-Cytoplasmic Protein Is Expressed in Thymus and Spleen and Is Induced in HL-60 Cells by TPA. The PTPε-cytoplasmic protein was detected when blots prepared from extracts of mouse spleen and thymus were analyzed with anti-PTPε polyclonal antiserum (7). These experiments revealed the presence of a protein of ≈72 kDa, in good agreement with the predicted molecular mass of the PTPε-cytoplasmic protein, 74,675 Da (Fig. 6). The same protein was detected in extracts of HL-60 cells and was significantly up-regulated in these cells in response to TPA treatment. Consistent with the absence of PTPε-cytoplasmic mRNA from brain (Fig. 1), this protein was not detected in protein extracts from this organ (Fig. 6, lane 5). The 72-kDa protein was not detected when the experiments were repeated with immune serum in the presence of the immunizing peptide (Fig. 6, lane 6) or with preimmune serum (not shown), further strength-
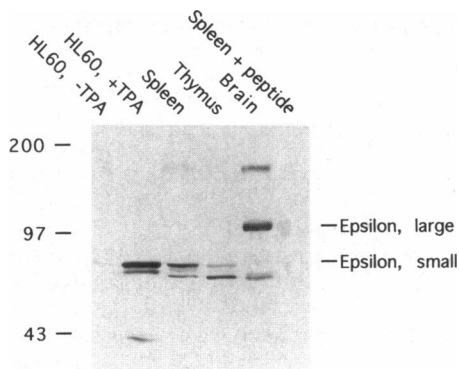

FIG. 7. The PTPε-cytoplasmic protein is cytosolic. (*Left*) HL-60 cells were either naive (lanes −) or were treated with TPA (lanes +) for 24 hr. Cells were fractionated into nuclear (lanes Pellet) and nonnuclear supernatant (lanes Sup.) fractions and analyzed with the anti-PTPε antiserum. (*Right*) The nonnuclear fraction was further separated into soluble (S100) and particulate (P100) fractions and analyzed as above. The position of the PTPε-cytoplasmic protein is denoted by "Epsilon, small." The same extracts were analyzed for the cytoplasmic GAP protein as a positive control. Molecular mass markers are in kDa.

ening its identification as the PTPε-cytoplasmic molecule. Interestingly, no PTPε-cytoplasmic protein was detected in extracts of NIH 3T3 cells after TPA treatment (not shown), possibly indicating a measure of translational control of PTPε-cytoplasmic expression.

The PTPε-Cytoplasmic Protein Is a Nontransmembranal Molecule. To demonstrate that the PTPε-cytoplasmic protein is a nontransmembranal molecule, we performed a series of subcellular fractionations of TPA-treated and naive HL-60 cells. Cells were lysed with the nonionic detergent Nonidet P-40, and the extracts were separated by centrifugation into nuclear and nonnuclear (i.e., cytoplasmic and cellular membrane-derived) fractions. Analysis of these fractions showed that the PTPε-cytoplasmic signal was present exclusively in the nonnuclear fraction (Fig. 7 *Left*).

HL-60 cells were then treated with TPA and lysed by Dounce homogenization without addition of detergents. The nonnuclear fraction obtained in this manner was further subdivided by centrifugation into membranal and cytosolic fractions and analyzed as above. The PTPε-cytoplasmic protein was present almost exclusively in the cytoplasmic fraction (Fig. 7 *Right*). The same results were obtained with HL-60 cells that had not been treated with TPA, indicating that TPA treatment does not change the subcellular localization of the PTPε-cytoplasmic isoform. As a positive control, the subcellular distribution of the cytoplasmic protein ras-GAP was also determined. A weak PTPε-cytoplasmic signal was detected in the membranal fraction of TPA-stimulated HL-60 cells (Fig. 7 *Right*, lane 5). The amounts of the PTPε-cytoplasmic isoform present in the nonstimulated cells are low, and a correspondingly small fraction of membrane-associated PTPε-cytoplasmic protein in these cells would probably be undetectable. Therefore, we cannot totally rule out the possibility that a small fraction of the PTPε-cytoplasmic protein is associated with the cellular membrane.

DISCUSSION

We have described a second nontransmembranal isoform of PTPε whose expression pattern, and hence physiological role, is different from that of the known transmembranal isoform of PTPε. Examination of its expression pattern suggests that the role of the PTPε-cytoplasmic isoform may ultimately be found in the context of the hematopoietic system.

Examination of the sequence of the PTPε-cytoplasmic cDNA reveals that it differs from the PTPε-transmembranal

FIG. 6. Detection of the PTPε-cytoplasmic protein in extracts of cells and mouse organs. PTPε was detected with a polyclonal anti-PTPε serum raised against a peptide from a region common to both PTPε isoforms (7). Protein in the lane marked "spleen + peptide" was hybridized with anti-PTPε antiserum in the presence of excess immunizing peptide. The positions of the transmembranal and cytoplasmic isoforms ("Epsilon, large" and "Epsilon, small") are indicated. Molecular mass markers are in kDa.
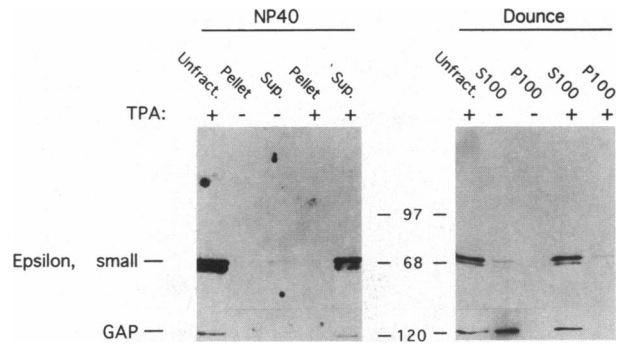
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cDNA only at its 5' end. This fact together with the existence of only one PTPε gene, as judged by DNA blot and by mapping studies (not shown), indicates that the PTPε gene contains two alternate, isoform-specific 5' exons and promoters. The decision regarding which isoform is to be expressed begins therefore at the level of transcription initiation. A system of this type is inflexible in the sense that neither of the two PTPε proteins would be a precursor of the other and provides additional support for the existence of a separate physiologic function for each isoform. The fact that the presence of PTPε mRNA in *myc*-based mammary tumor cell lines (7) or in fibroblasts (see above) is not invariably followed by PTPε protein expression indicates that expression of PTPε is regulated also at the translational level.

Their different amino termini notwithstanding, the two isoforms of PTPε are otherwise identical. Both have the same catalytic domains and adjacent sequences, indicating that they should be inherently capable of dephosphorylating the same substrates and interacting with the same effectors. How then can each isoform have a distinct physiological role? We propose that the differences in their subcellular localization and tissue specificity may cause each of the PTPε isoforms to encounter different substrates and interacting molecules, controlling function through substrate and effector availability. A similar argument has been suggested in explaining the differences in the phenotypes observed upon overexpression of T-cell PTPase and a carboxyl-terminally truncated form of the enzyme, each of which is located in a different cellular compartment (26). Alternatively, the transmembranal form may be capable of responding to extracellular signals, while the unique amino terminus of the cytoplasmic form may serve in a regulatory function. Examples of noncatalytic sequences affecting the catalytic properties of PTPases, as in the case of the cytoplasmic PTPase PTP1C (27, 28), have been previously described.

The existence of alternative forms of mRNA is fairly common among PTPases. Alternative splicing produces several forms of CD45 (reviewed in ref. 29), and splicing variants have been described for PTPases α (30, 31), β (6), and PTP1D (32), among others. Alternative PTPase mRNAs that share a common 5' end but differ in their 3' ends have also been described (33–37). Several growth factors induce alternative splicing of PTP1B mRNA, retaining its last intron and thereby altering the C terminus of the mature protein (38). PTPε appears somewhat unique in that the two PTPε mRNAs appear to arise through use of alternate 5' exons and promoters. Finally, the cytoplasmic from of PTPε is unique also in that it is the first PTPase shown to contain two catalytic domains, which heretofore were thought to be characteristic only of transmembranal PTPases.

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