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# Cloning and expression of a widely expressed receptor tyrosine phosphatase

(surface receptors/dephosphorylation/signal transduction)

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ABSTRACT **We describe the identification of a widely expressed receptor-type (transmembrane) protein tyrosine phosphatase (PTPase; EC 3.1.3.48). Screening of a mouse brain cDNA library under low-stringency conditions with a probe encompassing the intracellular (phosphatase) domain of the CD45 lymphocyte antigen yielded cDNA clones coding for a 794-amino acid transmembrane protein [hereafter referred to as *r*eceptor *p*rotein *t*yrosine *p*hosphatase α (R-PTP-α)] with an intracellular domain displaying clear homology to the catalytic domains of CD45 and LAR (45% and 53%, respectively). The 142-amino acid extracellular domain (including signal peptide) of R-PTP-α is marked by a high serine/threonine content (32%) as well as eight potential N-glycosylation sites but displays no similarity to known proteins. Genetic mapping assigns the gene for R-PTP-α to mouse chromosome 2, closely linked to the *Il-1a* and *Bmp-2a* loci. The corresponding mRNA (3.0 kilobases) is expressed in most murine tissues and most abundantly expressed in brain and kidney. Antibodies against a synthetic peptide of R-PTP-α identified a 130-kDa protein in cells transfected with the R-PTP-α cDNA.**

The identification of several growth factor receptors and retroviral oncogenes as tyrosine-specific protein kinases indicated that protein phosphorylation on tyrosine residues plays a key role in cellular growth control. This notion has recently received firm support by the observation that the level of tyrosine phosphorylation of enzymes thought to play an important role in signal transduction (such as phospholipase C) correlates well with their increased activity upon growth factor stimulation, thus establishing a functional role for tyrosine phosphorylation. The mechanisms leading to changes in activity of tyrosine kinases are best understood in the case of receptor-type tyrosine kinases with a transmembrane topology. In this case, the binding of specific ligands to the extracellular domain of members of receptor-type tyrosine kinases is thought to induce their oligomerization leading to an increase in tyrosine kinase activity and activation of the signal transduction pathways. Deregulation of kinase activity through mutation or overexpression is a well-established mechanism for cell transformation (1, 2).

Despite the historical emphasis on the study of kinase activity, several lines of reasoning warrant a closer study of the phosphatases involved in control of phosphotyrosine metabolism. (*i*) Elevation of cellular phosphotyrosine contents could occur through mechanisms not involving the mutational activation of a tyrosine kinase itself. It could be also caused through mutation of the substrate or through general decreases in cellular phosphatase activity, especially in view of the normally high turnover rate of cellular tyrosine-phosphate (3). The latter possibility has indeed received support through the use of tyrosine phosphatase inhibitors, which have been shown to confer a reversible transformed phenotype on cells (4). In view of this observation, tyrosine phosphatases could be considered as potential recessive oncogenes. (*ii*) Dephosphorylation of tyrosine residues can by itself function as an important regulatory mechanism. Dephosphorylation of a C-terminal tyrosine residue has been shown to activate tyrosine kinase activity in the case of the src family of tyrosine kinases (5), whereas tyrosine dephosphorylation has been suggested to be an obligatory step in the mitotic activation of the maturation-promoting factor kinase (6). These observations make it mandatory to better understand the mechanisms that regulate tyrosine-phosphatase activity. (*iii*) Mutant analysis in primitive eukaryotes has established crucial roles for serine-phosphatases in cellular physiology (7).

The precise nature of the protein tyrosine phosphatases (PTPases; EC 3.1.3.48) involved has until recently been unclear, primarily due to severe difficulties encountered in their purification (8). Pioneering studies have recently identified a class of cytoplasmic phosphatases bearing high substrate affinity as well as strict specificity for phosphotyrosyl residues and therefore likely to be of physiological relevance (9, 10). Remarkably, in addition to soluble proteins, the same family appears to include transmembrane protein members with N-terminal extracellular domains, thus establishing a striking analogy with the tyrosine kinase family (11–14). It is therefore reasonable to suggest that, in analogy to receptor tyrosine kinases, ligands in the extracellular environment could control the activity of this subclass of PTPases. This notion has received some support by studies on the CD45 molecule (15).

Taken together, the above observations suggest that PTPases may play an important role in cellular control mechanisms, as potential (anti)oncogenes and as effectors in a mechanism of transmembrane signaling. We have undertaken a search for additional PTPases potentially involved in such processes and describe here the identification of a widely expressed member of this family with transmembrane topology.§

## MATERIALS AND METHODS

**Library Screening.** A mouse BALB/c brain cDNA library in λgt11 (kindly provided by Y. Citri, The Weizmann Institute) was screened at relaxed stringency using as a probe a 2400-base-pair (bp) *Bgl* II–*Acc* I fragment representing the intracellular and transmembrane domains of the human T200 glycoprotein (16). Out of $10^6$ clones, 51 positives were picked, selected, and characterized by restriction enzyme mapping. *Eco*RI fragments of 0.95, 1.6, and 0.3 kilobases (kb) isolated

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Abbreviations: PTPase, protein tyrosine phosphatase; R-PTP-α, receptor protein tyrosine phosphatase α; RI, recombinant inbred.

§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M34668).

from the phage clone containing the longest insert (λ-109) were subcloned into the Bluescript KS plus and minus vectors. A series of nested deletions were generated by making use of restriction sites common to the cloned cDNA fragments and the polylinker region of the plasmid vector. The individual restriction sites used are indicated in Fig. 1 *Lower*. Single-stranded DNA was prepared from these constructs, and used as a template for sequence analysis using the dideoxynucleotide chain-termination method (Sequenase, United States Biochemical). All regions were sequenced on both strands.

**Chromosomal Localization.** STS/A, O20/A, CXS, and OXA recombinant inbred (RI) mice and CXB RI strains N, O, P, Q, and R were a gift from J. Hilgers (The Netherlands Cancer Institute). All other inbred mice were purchased from The Jackson Laboratory. Backcross animals were bred at New York University with inbred progenitors obtained from The Jackson Laboratory. Spleen genomic DNA from the AKXD, AKXL, BXD, BXH, and SWXL RI strains and from CXB RI strains D, E, G, H, I, J, and K was purchased from the DNA Resource at The Jackson Laboratory. For all other mice, genomic DNA was prepared from crude liver nuclei by a standard sequence of protease digestion, phenol and chloroform extraction, and ethanol precipitation. Mouse genomic DNAs were subjected to Southern blotting analysis by slight modifications of standard procedures, exactly as described (17). A 1.8-kb *Eco*RI fragment corresponding to the intracellular phosphatase domains of *r*eceptor *p*rotein *t*yrosine *p*hosphatase α (R-PTP-α) and a 0.7-kb *Sac* II–*Eco*RI fragment corresponding to its extracellular and transmembrane domains were cloned into the Bluescript KS vector, yielding plasmids p109 and p923, respectively.

**Northern Analysis.** Poly(A)$^+$ RNA was prepared from adult mouse tissues and cell lines by oligo(dT) selection as described (18), fractionated (5 μg per lane) on a formaldehyde-containing gel, transferred to nitrocellulose (Hybond C, Amersham) using standard procedures, and probed with the entire R-PTP-α cDNA.

**Expression of the R-PTP-α Protein.** The entire cDNA insert from phage λ-109 was released as one fragment from the phage using partial *Eco*RI digestion and cloned into the Bluescript KS vector. A cDNA fragment lacking most of the untranslated leader sequence (starting from the *Sac* II site at position 226; see Fig. 1 *Lower*) was subcloned into the simian virus 40 promoter-driven pLSV vector (19), and the resulting plasmid DNA (pLSV-PTP-α) was transfected into COS cells using the DEAE-dextran method (20).

**Antibody Preparation and Immunoprecipitation.** Rabbits were injected with a synthetic peptide corresponding to the predicted C terminus of the R-PTP-α protein (residues 777–794) coupled to bovine serum albumin using 1-ethyl-3-(dimethylaminopropyl)carbodiimide as a coupling reagent. Antigen was injected intradermally and subcutaneously in an emulsion of 1 mg of peptide and complete Freund's adjuvant. Three booster injections were given at 2- to 3-week intervals with 0.5 mg of peptide and incomplete adjuvant. Metabolic [$^{35}$S]methionine labeling, cell extract preparation (60 hr after transfection), and indirect immunoprecipitation using protein A Sepharose were performed using standard procedures (19).

## RESULTS

**Isolation and Analysis of R-PTP-α cDNA Clones.** To search for further PTPases, we screened at low stringency a mouse brain cDNA library, using as a hybridization probe the intracellular domain of human CD45 containing two tandem PTPase domains (11, 12, 16). The result of the nucleotide sequence analysis of the longest phage insert (λ-109) is shown in Fig. 1. Conceptual translation of the cDNA sequence reveals the existence of a major open reading frame of 794 amino acids, assuming that translation initiates at nucleotides 259 (an in-frame stop codon is present 60 nucleotides upstream). The putative initiation methionine codon is embedded in a relatively standard environment for initiation of translation (21) and is followed by a characteristic hydrophobic stretch of amino acids that probably function as a signal peptide. A second hydrophobic stretch is found between amino acids 143 and 166 and is followed by a series of highly charged residues, consistent with the stop-transfer signals found to be associated with many membrane-spanning domains. The predicted intracellular domain of the protein consists of two tandem repeats having 44% sequence identity between each other (residues 259–486 and 552–776). Each of these repeats displays significant sequence identity with the intracellular catalytic domains of the previously described transmembrane PTPases CD45 (16) and LAR (13) (45% and 53%, respectively, amino acid sequence identity). By contrast, the EMBL and GenBank data bases contain no significant homology to known sequences of the putative extracellular domain of the encoding protein. Features of the extracellular domain include a uniquely high content of serine and threonine residues (>32%), the absence of cysteine residues, and the presence of eight potential N-linked glycosylation sites.

We conclude that the isolated cDNA encodes an additional member of the transmembrane PTPase family having a unique type of extracellular domain. We propose the name R-PTP-α for this protein.

**Chromosomal Localization of the Mouse R-PTP-α Gene.** Southern blotting analyses of genomic DNA from inbred strains of mice revealed two useful restriction length variants, one visualized with a probe corresponding to the intracellular domain of R-PTP-α (p109) and one visualized with an extracellular and transmembrane domains probe (p923). Together, these variants allowed definition of three allelic forms of R-PTP-α among the 10 inbred strains of mice surveyed and inheritance of these alleles in RI mice was scored (Table 1). Comparison of the strain distribution patterns observed for R-PTP-α with those previously observed for other markers of known chromosomal location in these mice indicated close linkage between R-PTP-α and interleukin 1 (*Il-1a*) on chromosome 2 (3 recombinant strains, AKXD-11, CXB-O, and CXS-7, among 89 examined). This fraction of recombinant strains has <1 chance in 100,000 of occurring as a chance event were the loci unlinked (22, 23) and indicates a distance between the genes of 0.9 centimorgan (cM) (95% confidence limits, 0.2–0.6 cM) (24, 25). Following the inheritance of R-PTP-α, *Il-1a*, and *a* (nonagouti) among 150 progeny of reciprocal backcross between the inbred strains C57BL/6J and SWR/J confirmed the linkage of R-PTP-α and *Il-1a* and suggested an order for the two genes. Fourteen of the progeny were recombinant between R-PTP-α and *a*, and one was recombinant R-PTP-α and *Il-1a*. If the locus order is centromere, *Il-1a*, R-PTP-α, *a*, these results require the occurrence of no double crossovers; alternative orders require 1 or 14 such events and, evaluated according to the method of Bishop (26), are at least 9.5-fold less likely. The distance between *Il-1a* and R-PTP-α, 0.6 cM (95% confidence limits, 0.1–2.4 cM), agrees with in-sampling fluctuation with that from the RI strain data. Comparison of these results with ones recently obtained for *Bmp-2a* (bone morphogenic protein 2a) (27) suggests that the two genes may be closely linked, although there is no obvious structural homology between them.

**Expression of the R-PTP-α mRNA.** Northern analysis (Fig. 2) reveals a wide pattern of expression of the R-PTP-α mRNA. A 3.0-kb mRNA is present in all tissues examined, except spleen, with brain and kidney showing the highest levels of expression. An mRNA of similar size could also be observed in NIH 3T3 mouse fibroblast clones 2.2 (28) and (prepro-B lymphoid) BAF (29) cell line (Fig. 2). Shorter

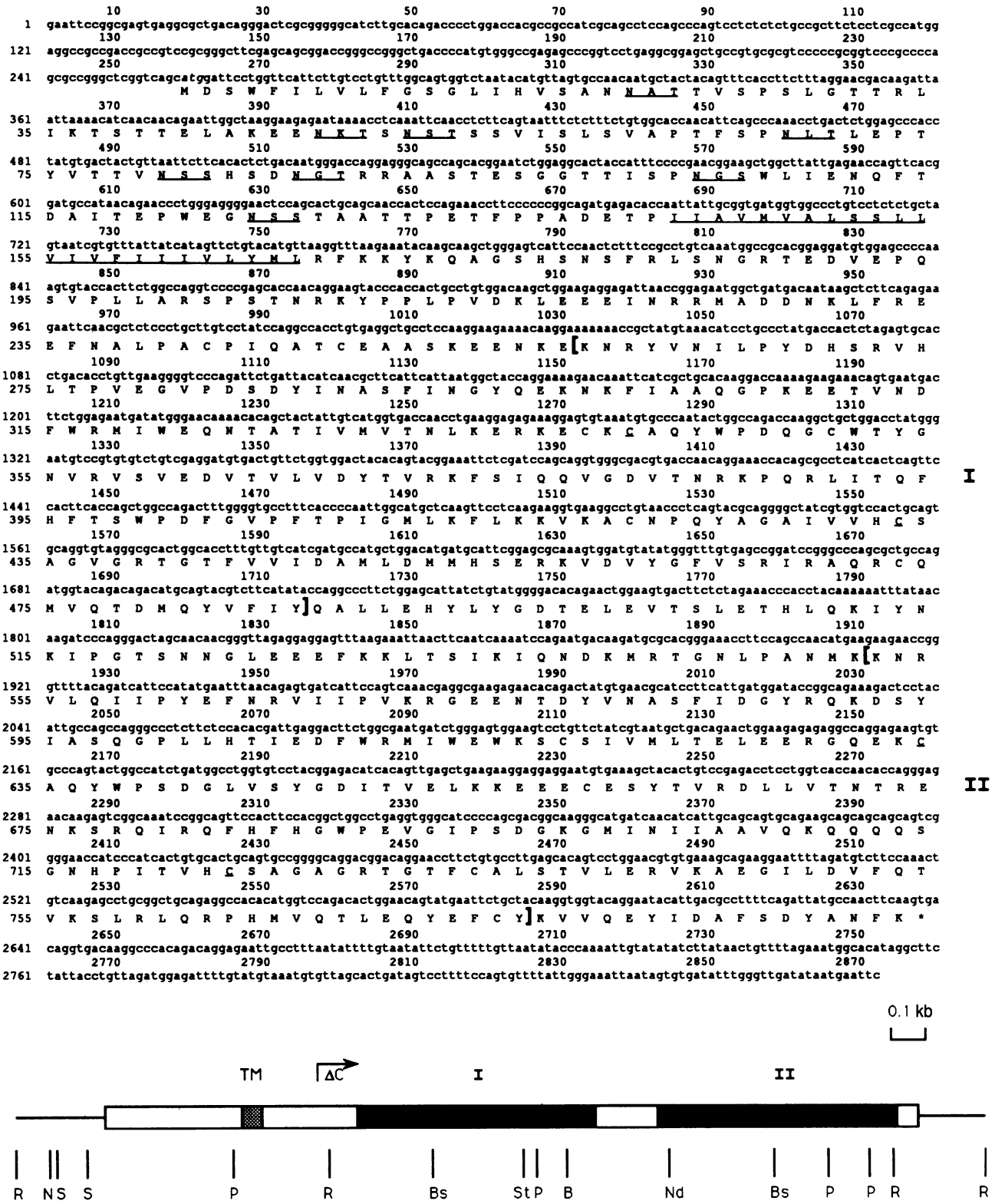


FIG. 1. Predicted primary structure of *r*eceptor *p*rotein *t*yrosine *p*hosphatase α (R-PTP-α). (*Upper*) Sequence of the phage λ-109 cDNA insert and predicted R-PTP-α protein sequence (the standard one-letter code is used). The initiation ATG is shown in italics, and the stop codon is indicated by an asterisk. The putative transmembrane domain (amino acids 143–166) is underlined as well as the potential N-linked glycosylation sites in the extracellular domain. The borders of homology between the tandemly repeated PTPase domains (I and II) are indicated by square brackets. Cysteine residues conserved in the catalytic domain of all known R-PTPases are also underlined. (*Lower*) Schematic structure of λ-109 cDNA clone containing the R-PTP-α coding sequence. R-PTPase domains I and II are indicated as black boxes; the transmembrane domain is shaded. The start of the N-terminally truncated PTP-ΔC protein mentioned in Fig. 3 is indicated by the arrow (amino acid 214). The positions of restriction sites used for generating nested deletions for sequencing are indicated. TM, transmembrane domain; B, *Bam*HI site; Bs, *Bst*EII site; N, *Nco* I site; Nd, *Nde* I site; P, *Pst* I site; R, *Eco*RI site; S, *Sac* II site; St, *Stu* I site.

exposures of the Northern blot clearly show that a second mRNA species of very similar size (3.2 kb) is also present in several tissues (e.g., brain) in lower amounts. In addition, the data suggest that, although we do not observe a poly(A) tail

Table 1. Restriction fragment length variants detected by R-PTP-α probes

| Allele | p109, kb | p923, kb | Strain |
|---|---|---|---|
| *a* | 9.4 | 5.9 + 4.2 | BALB/cJ |
| | | | CXB-D, H, N, P, Q; CXS-4, 5, 7, 9, 11, 12, 14 |
| *b* | 6.5 | 4.2 + 1.8 | C57BL/6J, C57L/J, DBA/2J |
| | | | AKXD-1, 2, 3, 7, 10, 13, 15, 18, 21, 23, 25 |
| | | | AKXL-5, 6, 7, 9, 13, 14, 16, 21, 25, 29, 38 |
| | | | BXH-2, 3, 7, 9, 11, 19; BXJ-1, 2 |
| | | | CXB-E, G, I, J, K, Q, R; SWXL-7, 14, 15, 17 |
| *c* | 6.5 | 5.9 + 4.2 | C3H/HeJ, 020/A, AKR/J, SWR/J, SJL/J, STS/A |
| | | | AKXD-6, 8, 9, 11, 12, 16, 20, 22, 24, 26, 27, 28 |
| | | | AKXL-8, 12, 17, 19, 24, 28, 37 |
| | | | BXH-4, 6, 8, 10, 12, 14; CXS-1, 2, 3, 6, 8, 10, 13 |
| | | | SWXL-4, 12, 16 |

Liver or spleen genomic DNA digested with *Taq* I restriction endonuclease was analyzed by Southern blotting. All RI strains were also typed for alleles of *Il-1a* (17); the three RI strains recombinant between the two loci are underlined.

or a poly(A) signal at the 3′ end of the cDNA sequence, the isolated cDNA clone (2872 nucleotides) closely matches the full length of the mRNA.

**Transient Expression of the R-PTP-α Protein.** To determine the size of the mature protein, we cloned the R-PTP-α cDNA with the exception of most of the untranslated leader (see *Materials and Methods* for details) into the pLSV vector (19) under the control of the simian virus 40 promoter, yielding the expression vector pLSV-PTP-α. The vector was transfected into COS cells, and 60 hr later [$^{35}$S]methionine-labeled total cell extracts were prepared for immunoprecipitation, using an antiserum (2A) directed against a synthetic peptide corresponding to the C terminus of the R-PTP-α protein. As seen in Fig. 3, the antiserum recognized several bands, one of which, a diffuse band of 130 kDa (arrow), was only present in immunoprecipitates from transfected cells (lane 5) but not from mock-transfected cells (lane 3) (transfected with pLSV without the R-PTP-α cDNA). Precipitation could be competitively inhibited by the peptide used for immunization (lane 6). The difference between the predicted (88 kDa) and observed (130 kDa) molecular masses for the R-PTP-α protein probably results from its extensive glycosylation. As an additional control for the specificity of the antiserum, we also transfected COS cells with an N-truncated version of the R-PTP-α cDNA (starting at amino acid 214, and thus lacking the transmembrane and extracellular domain) in the same vector. In this case, a protein with an apparent molecular mass of 55 kDa appeared in immunoprecipitates from cells transfected with this vector, which was again competitively inhibited by the antigenic peptide (lanes 7 and 8). The higher abundance of the truncated protein as compared to the mature R-PTP-α protein was a consistent observation over several independent transfection experiments.

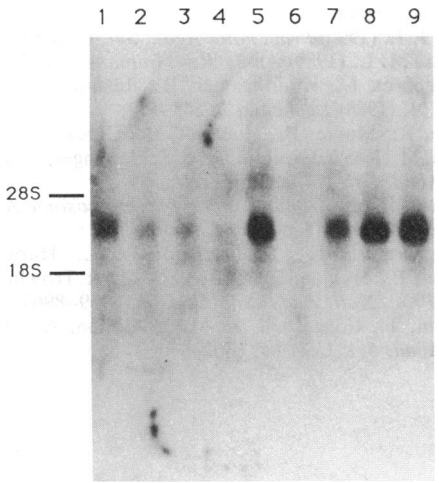


FIG. 2. Expression of the R-PTP-α mRNA. Northern blot analysis of poly(A)$^+$ RNA from mouse tissues and cell lines. Lanes: 1, kidney; 2, lung; 3, heart; 4, stomach; 5, brain; 6, spleen; 7, liver; 8, NIH 3T3 fibroblast cell line (28); 9, BAF prepro-B lymphoid cell line (29).

## DISCUSSION

It has recently been recognized that tyrosine-specific protein phosphatases comprise a separate family of proteins with different enzymatic properties from, and no common ancestry with, serine/threonine-specific phosphatases. In addition to soluble and possibly palmitoylated enzymes (30, 31), two groups of PTPases have been described with a transmembrane topology. The leukocyte common antigen (CD45, L-CA, T200, Ly-5) (32) comprises a group of membrane

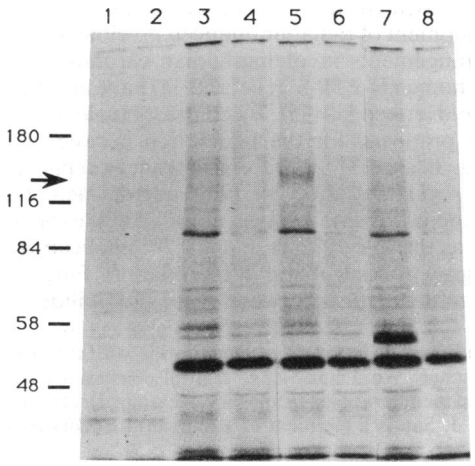


FIG. 3. Immunoprecipitation of the R-PTP-α protein. Transiently transfected COS cells were subjected to metabolic labeling with [$^{35}$S]methionine and immunoprecipitation using either preimmune serum (lanes 1 and 2) or antipeptide antiserum (2A) (lanes 3–8) against the R-PTP-α protein in the absence or presence of 100 μg of the immunizing peptide. Sizes of molecular mass markers are shown in kDa. The arrow marks the position of the 130-kDa R-PTP-α protein in lane 5. Plasmids used for transfection: pLSV, expression vector without insert (control); pLSV-PTP-α, expressing the entire R-PTP-α cDNA; PTP-ΔC, expressing only the cytoplasmic portion of R-PTP-α (amino acids 214–794). Lanes: 1, pLSV, preimmune serum; 2, pLSV-PTP-α, preimmune serum; 3, pSLV, antiserum 2A; 4, pLSV, antiserum 2A in the presence of synthetic peptide; 5, pLSV-PTP-α, antiserum 2A; 6, pLSV-PTP-α, antiserum 2A in the presence of synthetic peptide; 7, pLSVΔC, antiserum 2A; 8, pLSVΔC, antiserum 2A in the presence of synthetic peptide.

glycoproteins expressed exclusively in hemopoietic cells, derived from a common gene by alternative splicing events involving the N terminus of the proteins. Whereas the restricted and lineage-specific expression of the various CD45 isoforms suggests that their function is linked to specialized immunological events, a molecule isolated by virtue of its homology to CD45, LAR, was shown to have a significantly wider pattern of expression and even has a *Drosophila* homologue (13, 14).

In the present study, we describe the identification of a receptor-like PTPase, R-PTP-$\alpha$, showing a broad pattern of expression, suggesting that R-PTPases may have widespread functions beyond the regulation of lymphoid cell activity. Extensive studies using monoclonal antibodies directed against the extracellular domain of CD45 proteins have shown that cross-linking of R-PTPases can have profound effects on various cellular activities (15, 32). Since ligand-induced receptor clustering is a central event in transmembrane signaling by receptor tyrosine kinases (2), it can be postulated that extracellular putative ligands for R-PTPases would have the capacity to regulate the activity of R-PTPases *in vivo*. The variety of extracellular domains apparently joined to PTPase domains to form receptor-like proteins might then reflect the range of possible ligands able to act in such a manner.

Two major but probably simplified possibilities can be put forward for the function of R-PTPases. On the one hand, R-PTPases might have a broad specificity directed toward major tyrosine kinase substrates, with their different extracellular domains mainly allowing for different regulatory mechanisms. In this case, they might be expected to modulate the responsiveness of a cell to polypeptide growth factors acting through receptor protein tyrosine kinases. If, as is the case for protein tyrosine kinases, ligand binding leads to an activation of enzymatic activity, molecules such as R-PTP-$\alpha$ can be considered potential recessive oncogenes. For instance, deletion of portions of chromosome 2, on which R-PTP-$\alpha$ is located, has been described as an early event in the development of radiation-induced myeloid leukemia (33), and rearrangements involving human chromosome 20 (carrying the human R-PTP-$\alpha$ gene; ref. 34) have also been linked to lymphoid leukemias (35). By contrast (analogously to what has been proposed for the interaction between CD45 and c-lck; refs. 36 and 37), R-PTP-$\alpha$ might act to dephosphorylate negative regulatory sites in nonreceptor membrane-associated protein tyrosine kinases more widely expressed than lck, such as the Tyr-527 site in $pp60^{c\text{-}src}$, implicating it also in positive growth control and differentiation. Further work is required to distinguish between these possibilities.

This study was initiated as a collaboration with the laboratory of Dr. E. Fischer. We acknowledge fruitful discussions with Dr. E. Fischer and members of his laboratory, including Dr. D. Cool and Dr. N. Tonks. J. Sap is a recipient of a long-term EMBO fellowship.

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