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A family of receptor-linked protein tyrosine phosphatases in humans and *Drosophila*

(leukocyte common antigen/CD45/LAR/immunoglobulin superfamily/tyrosine phosphorylation)

MICHEL STREULI*†, NEIL X. KRUEGER*†, ALEX Y. M. TSAI*‡, AND HARUO SAITO*§¶

*Division of Tumor Immunology, Dana-Farber Cancer Institute, and Departments of †Pathology and §Biological Chemistry and Molecular Pharmacology, Harvard Medical School, and ‡Department of Periodontology, Harvard School of Dental Medicine, Boston, MA 02115

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ABSTRACT **To understand the regulation of cell proliferation by tyrosine phosphorylation, characterization of protein tyrosine phosphatases (PTPase; protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48) is essential. The human genes LCA (leukocyte common antigen) and LAR encode putative receptor-linked PTPases. By using consensus sequence probes, two additional receptor-linked PTPase genes, DLAR and DPTP, were isolated from *Drosophila melanogaster*. The extracellular segments of both DLAR and DPTP are composed of multiple immunoglobulin-like domains and fibronectin type III-like domains. The cytoplasmic region of DLAR and DPTP, as well as human LCA and LAR, are composed of two tandemly repeated PTPase domains. PTPase activities of immunoprecipitated LCA and LAR were demonstrated by measuring the release of phosphate from a ^{32}P-labeled [Tyr(*P*)]peptide. Furthermore, the cytoplasmic domains of LCA, LAR, DLAR, and DPTP, expressed in *Escherichia coli*, have PTPase activity. Site-directed mutagenesis showed that a conserved cysteine residue is essential for PTPase activity.**

Protein tyrosine phosphorylation by protein tyrosine kinases (PTKases; ATP:protein-tyrosine *O*-phosphotransferase, EC 2.7.1.112) is critically important in the regulation of cell growth and proliferation (1, 2). Although protein tyrosine phosphatases (PTPases; protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48) could be equally important in this regulation, their properties are not well characterized. Recently, the human placental PTPase 1B, a 35-kDa soluble enzyme, was purified and its amino acid sequence was partially determined (3, 4). Amino acid sequence comparison suggests that the cytoplasmic regions of two human transmembrane molecules, the leukocyte common antigen (LCA, also called CD45, Ly-5, T200, or B220) and LAR, which was isolated by cross-hybridization to a LCA cDNA probe, contain two tandemly repeated PTPase 1B-like domains (4–6). The expression of LCA is restricted to hematopoietic cells, whereas the expression of LAR seems to be mainly on cells of epithelial origin. PTPase activity of purified LCA has been demonstrated (7). The structures of LCA and LAR suggest that they are receptor-linked PTPases and raise the possibility that their phosphatase activities are controlled by their interaction with specific ligands. Thus, regulation of protein phosphorylation by PTPases may be as important as the regulation by PTKases. In this report, we describe two newly discovered *Drosophila* genes, DLAR and DPTP, that encode putative receptor-linked PTPases, and we demonstrate tyrosine-specific phosphatase activities of human LCA and LAR and *Drosophila* DLAR and DPTP.

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MATERIALS AND METHODS

Buffers. Lysis buffer: 20 mM Tris·HCl, pH 8.0/150 mM NaCl/1 mM EDTA/1% (vol/vol) Triton X-100/1 mM phenylmethylsulfonyl fluoride/2× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone). Lysozyme buffer: 33 mM Tris·HCl, pH 8.0/2.5 mM EDTA/10 mM 2-mercaptoethanol. 10× phosphatase buffer: 250 mM Hepes, pH 7.3/50 mM EDTA/100 mM dithiothreitol.

Isolation of cDNA Clones. A cDNA library made from 4- to 8-hr *Drosophila* embryos (8) was screened with the following degenerate oligonucleotides: PTP-I, GTICA(C/T)TG(C/T)TC(A/C/G/T)GC(A/C/G/T)GGIGT; PTP-II, GTICA(C/T)TG(C/T)AG(C/T)GC(A/C/G/T)GGIGT. A 5′ region probe was prepared from DLAR23, and another *Drosophila* cDNA library made from 3- to 8-hr embryos (9) was screened to isolate DLAR55. The nucleotide sequences were determined by the dideoxynucleotide chain-termination method (10) using modified T7 DNA polymerase (11).‖

Substrates for Phosphatase Assays. The synthetic peptide Raytide (Oncogene Science, Manhasset, NY) or angiotensin II (Sigma) was labeled at its tyrosine residue using [γ-^{32}P]ATP and v-*abl* tyrosine kinase (Oncogene Science). Partially dephosphorylated casein hydrolyzate (Sigma) was labeled at serine residues with [γ-^{32}P]ATP and the catalytic subunit of bovine heart cAMP-dependent protein kinase (Sigma).

Phosphatase Assay. The phosphatase assay mixture (50 μl) contains 5 μl of 10× phosphatase buffer, 5 μl of radioactive substrate (^{32}P-labeled [Tyr(*P*)]Raytide {[Tyr(^{32}P)]Raytide}, ^{32}P-labeled [Tyr(*P*)]angiotensin II {[Tyr(^{32}P)]angiotensin II}, or ^{32}P-labeled [Ser(*P*)]casein hydrolyzate {[Ser(^{32}P)]casein hydrolyzate}; 1–5 × 10^5 cpm), and 5 μl of sample to be assayed. After incubation at 30°C or 37°C for the indicated time, reactions were terminated by addition of 750 μl of charcoal mixture [0.9 M HCl/90 mM sodium pyrophosphate/2 mM NaH_2PO_4/4% (vol/vol) Norit A]. After centrifugation in a microcentrifuge, the radioactivity in 400 μl of supernatant was measured. Enzyme activity was expressed in arbitrary units where 1 unit is the amount of protein phosphatase needed to release 3.33% of the total phosphate per min.

Immunoprecipitates. Cells were washed in phosphate-buffered saline (PBS) and suspended in ice-cold lysis buffer at a concentration of 0.8–1.6 × 10^7 cells per ml. After 5 min on ice, nuclei were removed by centrifugation at 700 × *g* for 5 min. Preformed immune complexes between monoclonal antibodies and rabbit anti-mouse IgG were prepared as described

Abbreviations: PTPase, protein tyrosine phosphatase; PTKase, protein tyrosine kinase; LCA, leukocyte common antigen; N-CAM, neural cell adhesion molecule; aa, amino acid; FN, fibronectin.

¶To whom reprint requests should be addressed at: Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115.

‖The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M27699 and M27700).

(12). One milliliter of cell extract was mixed with the appropriate preformed immune complex and incubated at 4°C for 12 hr, sedimented by a 10-sec centrifugation in a microcentrifuge, washed four times with lysis buffer and then four additional times with PBS, and suspended in 50 μl of PBS.

Bacterial Extracts. Isopropyl β-D-thiogalactoside (final concentration, 1 mM) was added to exponentially growing cultures of *Escherichia coli* DHB4 [Δ*phoA Pvu* II *phoR* (F' *lacI*^{Q} *pro*)] (13) carrying plasmids, and cultures were shaken at 30°C for 2 hr. Cells in 1.5-ml cultures were precipitated in a microcentrifuge and resuspended in 200 μl of lysozyme buffer containing hen egg lysozyme (1.0 mg/ml). After 10 min at room temperature, cells were lysed by three cycles of freezing–thawing. Ten microliters of 0.2 M $MgCl_2$ and 5 μl of DNase I (5 mg/ml) were added to the lysates and incubated at 25°C for 20 min, followed by addition of 20 μl of 0.2 M EDTA and 26 μl of 10% Triton X-100, and incubated at 25°C for 10 min. Protein contents of the extracts were determined according to Bradford (14).

Plasmid Constructions and *in Vitro* Mutagenesis. For plasmid constructions, see Fig. 6. *In vitro* site-directed mutagenesis was done essentially according to Foss and McClain (15).

Other Methods. All other molecular biological procedures are according to standard methods (16).

RESULTS AND DISCUSSION

Isolation of *Drosophila* PTPase cDNA Clones. By comparing the amino acid sequences of LCA and LAR PTPase domains, a consensus amino acid sequence VHCSAGV was identified. A *Drosophila* embryo cDNA library (8) was screened with two sets of degenerate oligonucleotide probes corresponding to the consensus sequence. Two groups of cDNA clones, called DLAR and DPTP (Fig. 1), were identified that encode proteins with significant homology to the PTPase domains. The combined nucleotide sequence of DLAR23 and DLAR55 is 7630 base pairs (bp) and contains an open reading frame of 2029 amino acids (aa). The nucleotide sequence of DPTP12 is 4477 bp and contains an open reading frame of 1462 aa. The deduced amino acid sequences of DLAR and DPTP are shown in Fig. 2.

Structures of DLAR and DPTP Molecules. The N terminus of DLAR has a hydrophobic stretch characteristic of a signal peptide. The most likely cleavage site is between the 32nd and 33rd amino acids from the N terminus (17). The mature DLAR protein is a transmembrane protein with a 1345-aa extracellular region, a 25-aa transmembrane segment, and a 627-aa cytoplasmic region. The cytoplasmic region of DLAR, which is homologous to LAR (72% identity; Fig. 2*A*), is composed of two repeated PTPase domains (DLAR-Cy1 and -Cy2; Fig. 3). Although the similarity between the extracellular regions of DLAR and LAR is only 36%, the overall structures are very similar. As has been observed for LAR (6), the N-terminal region of DLAR is composed of three tandemly repeated immunoglobulin-like domains that have significant similarity to neural cell adhesion molecule (N-CAM) (18) (data not shown). These immunoglobulin-like domains are followed by nine repeated domains that share structural features of the fibronectin (FN) type III repeat unit (19) (data not shown). In contrast, the human LAR has only eight FN type III-like repeat units.

DPTP also encodes a transmembrane protein with a 23-aa signal peptide, a 782-aa extracellular region, an 18-aa transmembrane segment, and a 639-aa cytoplasmic region (Fig. 2*B*). The cytoplasmic region of DPTP is composed of two repeated PTPase domains (DPTP-Cy1 and -Cy2; Fig. 3). The N-terminal region of DPTP contains two tandemly repeated immunoglobulin-like domains, which are followed by two FN type III-like domains (data not shown).

The structures of DLAR, DPTP, and several related molecules are schematically shown in Fig. 4. The organizations of the extracellular regions of LAR, DLAR, and DPTP, which consist of several immunoglobulin-like domains and FN type III-like domains, are typical of a CAM family that includes N-CAM, fasciclin II, and L1 (18, 21, 22). Thus, it is possible that LAR, DLAR, and DPTP are cell adhesion receptors.

PTPase Activities of LCA and LAR. LCA is composed of several isoforms ranging in size between 220 and 180 kDa that are generated by tissue-specific splicing (5, 20, 23). The largest 220-kDa isoform of LCA was partially purified by immunoprecipitation from the human B-cell line Raji using monoclonal anti-LCA antibodies 2H4 or GAP8.3 (24, 25). To measure PTPase activities, immunoprecipitates were incubated with [Tyr(^{32}P)]Raytide, a modified gastrin peptide, and the amount of radioactivity released as inorganic phosphate was determined. As shown in Fig. 5*A*, immunoprecipitates using 2H4 or GAP8.3 had PTPase activity, whereas control immunoprecipitates (2G9 or W6/32) had no detectable phosphatase activity. The tyrosine specificity of the LCA phosphatase was assessed by assaying the same immunoprecipitates for protein serine phosphatase activity, using [Ser(^{32}P)]casein hydrolyzate as substrate. As Fig. 5*B* shows, none of the immunoprecipitates had detectable serine phosphatase activity. To test whether the smallest LCA isoform (180 kDa) has PTPase activity, a mouse B-cell line, 300-19, was transfected with a cDNA construct (pMT-LCA.1; Fig. 6*A*) that encodes the 180-kDa human LCA isoform, and cell-surface-expressed LCA was immunoprecipitated with the monoclonal anti-LCA antibody UCHL-1. Fig. 5*C* shows that the 180-kDa LCA isoform also has PTPase activity.

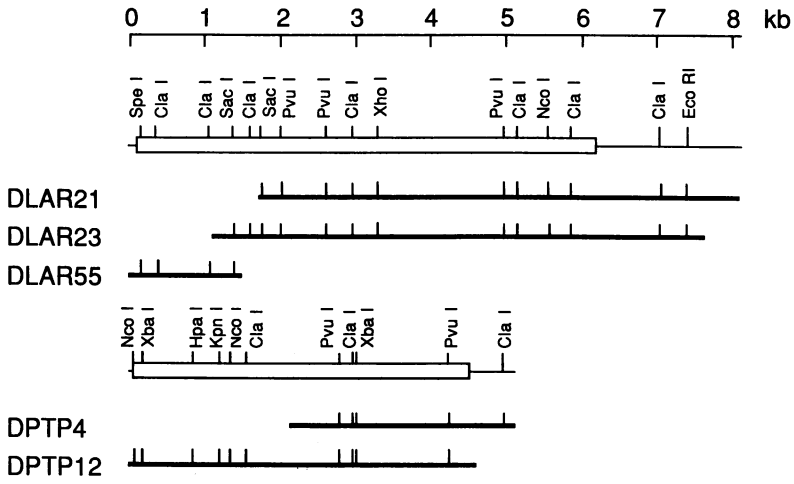

FIG. 1. Restriction maps of *Drosophila* cDNAs encoding two receptor-linked PTPases, DLAR and DPTP. Open boxes, protein encoding regions; thin lines, 5′ or 3′ noncoding regions. Below the restriction maps, thick lines indicate the sizes and extents of representative cDNA clones. kb, Kilobases.

FIG. 2. Amino acid sequences of the *Drosophila* receptor-linked PTPases, DLAR and DPTP. (*A*) DLAR amino acid sequence is aligned with the human LAR sequence (6). The standard one-letter code is used. Dashes in the LAR sequence indicate identical amino acids with DLAR; spaces in either sequence indicate gaps. The putative signal peptide and transmembrane peptide are underlined. Numbers to the right of each row refer to amino acid positions in the predicted mature protein. (*B*) DPTP amino acid sequence. Underlining and numbers are as in *A*.

To make it possible to purify the human LAR molecule by immunoprecipitation, we modified the LAR cDNA sequence so that it includes the epitope for the monoclonal antibody UCHL-1 (pMT-UC-LAR; Fig. 6*A*). The UCHL-1 epitope has been mapped to the N terminus of the 180-kDa LCA isoform (25). The modified LAR molecule expressed on transfected 300-19 cells and immunoprecipitated by the UCHL-1 antibody had PTPase activity (Fig. 5*D*).

Expression of Phosphatase Domains in *E. coli*. To study the PTPase domains in more detail, we inserted the cytoplasmic segments of the LCA, LAR, DLAR, and DPTP cDNA sequences into the prokaryotic expression vector pKKUC12 (Fig. 6*B*). The *E. coli* strain DHB4 (13) was transformed with these plasmids, and after induction by isopropyl β-D-thiogalactoside, extracts were prepared and assayed for PTPase activity. As Table 1 shows, cytoplasmic domains of LCA, LAR, DLAR, and DPTP expressed in *E. coli* have PTPase activities.

Mutations in Conserved Cysteine Residues. Because the placenta PTPase 1B is inhibited by various reagents that block cysteinyl groups, at least one cysteine is essential for PTPase activity (3). The comparison of the PTPase sequences in Fig. 3 indicates that there are two conserved cysteines in each PTPase domain. To examine whether these cysteines are essential for PTPase activity of LCA, we constructed mutants in which one or two of the conserved cysteine

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PTP  1B      ....NRNRYRDVSPFDHSRIKLHQEDND                                    YINASLIKMEEAQRSYILTQGPLPNTCGHFWEMVWEQKSRG
LCA  Cy1  ARKPFNQNKNRYVDILPYDYNRVELSEINGD                                AGSNYINASYIDGFKEPRKYIAAQGPRDETVDDFWRMIWEQKATV
LAR  Cy1  SNLEVNKPKNRYANVIAYDHSRVILTSIDGV                                PGSDYINANYIDGYRKQNAYIATQGPLPETMGDFWRMVWEQRTAT
DLAR Cy1  SNLEHNKSKNRYANVTAYDHSRVQLPAVEGV                                VGSDYINANYCDGYRKHNAYVATQGPLQETFVDFWRMCWELKTAT
DPTP Cy1  SDLKENACKNRYPDIKAYDQTRVKLAVINGL                                QTTDYINANFVIGYKERKKFICAQGPMESTIDDFWRMIWEQHLEI
LCA  Cy2  GNQEENKSKNRNSNVIPYDYNRVPLKHELEMSKESEHDSDESSDDDSDSEEPSKYINASFIMSYWKPEVMIAAQGPLKETIGDFWQMIFQRKVKV
LAR  Cy2  ANLPCNKFKNRLVNIMPYELTRVCLQPIRGV                                EGSDYINASFLDGYRQQKAYIATQGPLAESTEDFWRMLWEHNSTI
DLAR Cy2  ANLPCNKHKNRLVHILPYESSRVYLTPIHGI                                EGSDYVNASFIDGYRYRSAYIAAQGPVQDAAEDFWRMLWEHNSTI
DPTP Cy2  GENEENNMKNRSQEIIPYDRNRVILTPLPMR                                ENSTYINASFIEGYDNSETFIIAQDPFENTIGDFWRMISEQSVTT
           **  ** **** * ***  ** * * *                                     ************     ******** **  ***** ***

PTP  1B   VVMLNRVMEKGSLKCAQYWPQKEEKEMIFEDTNLKLTLISEDIKSYYTVRQLELENLTTQ     ETREILHFHYTTWP  DFGVPESPASFLNFL
LCA  Cy1  IVMVTRCEEGNRNKCAEYWPSMEEGTRAFGD  VVVKINQHKRCPDYIIQKLNIVNKKEK    ATGREVTHIQFTSWP  DHGVPEDPHLLLKLR
LAR  Cy1  VVMMTRLEEKSRVKCDQYWP  ARGTETCGL  IQVTLLDTVELATYTVRTFALHKSGSS     EKRELRQFQFMAWP  DHGVPEYPTPILAFL
DLAR Cy1  IVMMTRLEERTRIKCDQYWP  TRGTETYGQ  IFVTITETQELATYSIRTFQLCRQGFN     DRREIKQLQFTAWP  DHGVPDHPAPFLQFL
DPTP Cy1  IVILTNLEEYNKAKCAKYWPEKVFDTKQFGD  ILVKFAQERKTGDYIERTLNVSKNKANVGEEEDRRQITQYHYLTWK  DFMAPEHPHGIIKFI
LCA  Cy2  IVMLTELKHGDQEICAQYWG   EGKQTYGD  IEVDLKDTDKSSTYTLRVFELRHSKRK     DSRTVYQYQYTNWS  VEQLPAEPKELISMI
LAR  Cy2  IVMLTKLREMGREKCHQYWP  AERSARYQY  FVVDPMAEYNMPQYILREFKVTDARDG     QSRTIRQFQFTDWP  EQGVPKTGEGFIDFI
DLAR Cy2  VVMLTKLKEMGREKCFQYWP  HERSVRYQY  YVVDPIAEYNMPQYKLREFKVTDARDG     SSRTVRQFQFIDWP  EQGVPKSGEGFIDFI
DPTP Cy2  LVMISEIGD GPRKCPRYWA   DDEVQYDH  ILVKYVHSESCPYYTRREFYVTNCKID     DTLKVTQFQYNGWPTVDGEVPEVCRGIIELV
          ***** * *  * ** ****   *    **   * *          *  * *              *   ***** **  * **** *   * *

PTP  IB   FKV....
LCA  Cy1  RRVNAFSNF          FSGPIVVHCSAGVGRTGTYIGIDAMLEGLEAENKVDVYGYVVKLRRQRCLMVQVEAQYILIHQALVEYNQFGETEVN
LAR  Cy1  RRVKACNPL          DAGPMVVHCSAGVGRTGCFIVIDAMLERMKHEKTVDIYGHVTCMRSQRNYMVQTEDQYVFIHEALLEAATCGHTEVP
DLAR Cy1  RRCRALTPP          ESGPVIVHCSAGVGRTGCYIVIDSMLERMKHEKIIDIYGHVTCLRAQRNYMVQTEDQYIFIHDAILEAIICGVTEVP
DPTP Cy1  RQINSVYSL          QRGPILVHCSAGVGRTGTLVALDSLIQQLEEEDSVSIYNTVCDLRHQRNFLVQSLKQYIFLYRALLDTGTFGNTDIC
LCA  Cy2  QVVKQKLPQKNSSEGNKHHKSTPLLIHCRDGSQQTGIFCALLNLLESAETEEVVDIFQVVKALRKARPGMVSTFEQYQFLYDVIASTYPAQNGQVK
LAR  Cy2  GQVHKTKEQ        FGQDGPITVHCSAGVGRTGVFITLSIVLERMRYEGVVDMFQTVKTLRTQRPAMVQTEDQYQLCYRAALEYLGSFDHYAT
DLAR Cy2  GQVHKTKEQ        FGQDGPITVHCSAGVGRSGVFITLSIVLERMQYEGVLDVFQTVRILRSQRPAMVQTEDQYHFCYRAALEYLGSFDNYTN
DPTP Cy2  DQAYNHYKNNKN     SGCRSPLTVHCSLGTDRSSIFVAMCILVQHLRLEKCVDICATTRKLRSQRTGLINSYAQYEFLHRAIINYSDLHHIAES
           *                   ** ********** **     **    *  ***   *  ** **  ***** ** *   * **
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FIG. 3. Alignment of PTPase domain sequences of the placental PTPase 1B (4), LCA (5), LAR (6), DLAR, and DPTP. The positions where more than half of the sequences share an identical amino acid are indicted by asterisks and the positions where all sequences share an identical amino acid are highlighted by boldface type. Cy-1 and Cy-2 indicate first and second cytoplasmic PTPase domains of each molecule. The single-letter code is used.

residues at amino acid positions 737, 828, 1047, and 1144 were changed to serine and expressed the mutant LCA PTPases in *E. coli* DHB4. The results using the [Tyr(^{32}P)]Raytide substrate (Table 1) can be summarized as follows. The mutations at amino acid positions 737, 1047, and 1144 have no effect on the PTPase activity. On the other hand, the mutation at amino acid position 828 completely abolishes the PTPase activity. While it is possible that the 828S mutation affects both PTPase domains by changing protein conformation, a more plausible interpretation is that in our *in vitro* assay only the first PTPase domain has detectable activity, and for this activity Cys-828 is essential. One possible reason why the second PTPase domain of LCA has no activity in our *in vitro*

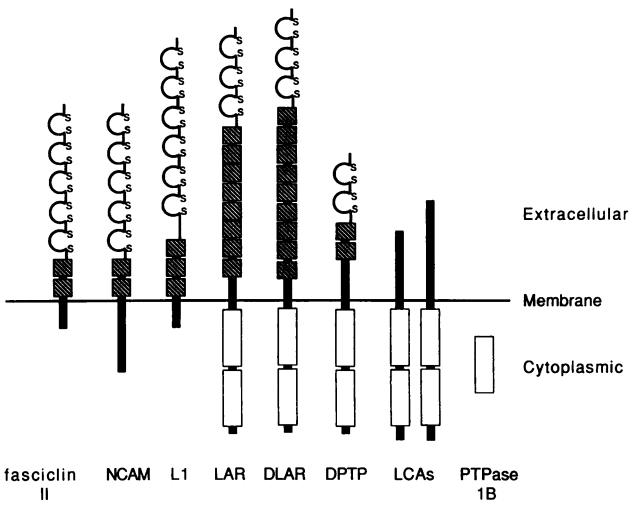

FIG. 4. Schematic comparison of the PTPase family and CAM family. The immunoglobulin-like, FN type III-like, and PTPase domains are represented by characteristic disulfide-bonded structures, small hatched boxes, and large open boxes, respectively. Thick vertical lines indicate the segments of protein that are unique. Two of the several LCA isoforms generated by alternative splicing are shown (5, 20).

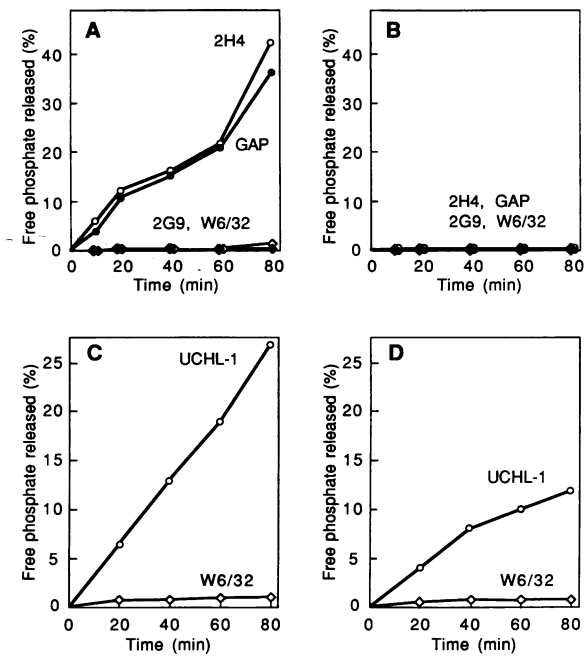

FIG. 5. PTPase activities of LCA and LAR. (*A*) The 220-kDa LCA isoform was purified by immunoprecipitation from the B-cell line Raji using the anti-human LCA monoclonal antibodies 2H4 or GAP8.3. Control antibodies are isotype-matched 2G9 and W6/32. PTPase activity in immunoprecipitates was assayed with [Tyr(^{32}P)]Raytide at 30°C for the indicated time. The amount of radioactivity released as inorganic phosphate is expressed as the percentage of total input radioactivity. (*B*) The same immunoprecipitates used in *A* were assayed for serine phosphatase activity by using [Ser(^{32}P)]casein hydrolyzate. (*C*) The 180-kDa LCA isoform was immunoprecipitated from the mouse B-cell line 300-19 transfected with pMT2-LCA.1 (Fig. 6*A*) using the anti-human LCA monoclonal antibody UCHL-1 and assayed for PTPase activity as described in *A*. The control antibody was W6/32. (*D*) The modified LAR molecule, UC-LAR, was immunoprecipitated from 300-19 cells transfected with pMT2-UC-LAR (Fig. 6*A*) using UCHL-1, and PTPase activity was assayed. The control antibody was W6/32.

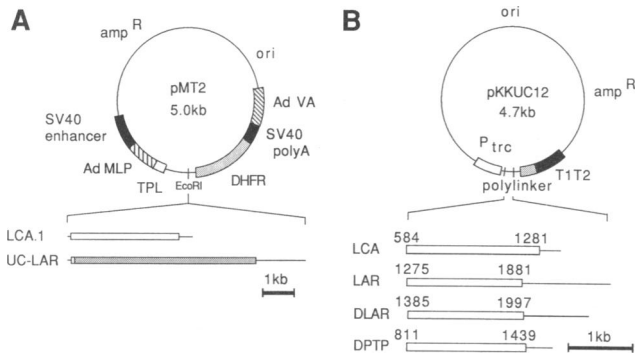

FIG. 6. (*A*) Schematic representation of plasmids used to transfect the mouse B-cell line 300-19. The complete LCA.1 cDNA (5) or the hybrid LAR cDNA sequence (UC-LAR) that contains the very 5′ end of LCA.1 and most of the LAR cDNA (6) was inserted into the eukaryotic expression vector pMT2 (26) at its unique *Eco*RI site. Open box, coding region derived from LCA.1; shaded box, coding region derived from the LAR cDNA. Ad MLP, adenovirus major late promoter; TPL, tripartite leader; Ad VA, adenovirus virus-associated genes; DHFR, dihydrofolate reductase; SV40, simian virus 40; amp^R, ampicillin resistance; kb, kilobase. (*B*) Schematic representation of plasmids used to transform *E. coli* DHB4. pKKUC12 was modified from the prokaryotic expression vector pKK233-2 (27) by adding a polylinker sequence with several extra restriction sites (*Kpn* I, *Bgl* II, *Sma* I, *Xba* I). The cytoplasmic domains of PTPase cDNAs were inserted within the polylinker so that the coding sequences are in-frame with the initiation ATG codon within the P_{trc} promoter region. Horizontal boxes and thin lines, protein encoding regions and 3′ noncoding regions, respectively. Numbers above the boxes indicate the amino acid positions of the segments. P_{trc}, trc promoter; T1T2, transcription terminators.

assay is that the [Tyr(^{32}P)]Raytide is not a suitable substrate for the second PTPase domain. To test this possibility, we used another PTPase substrate, [Tyr(^{32}P)]angiotensin II. However, there was no qualitative difference between Raytide and angiotensin II (Table 1). This result weakens, but does not exclude, the possibility that substrate specificity is the reason why the second PTPase domain is inactive. The trivial explanation that the second PTPase domain of LCA is intrinsically inactive seems unlikely, because the amino acid sequences of this domain are strongly conserved between mouse, rat, and human. Finally, it is possible that the activity of the second PTPase domain is specifically regulated (for example, by ligand binding).

Table 1. PTPase activity in extracts of *E. coli* DBH4 cells transformed with expression plasmids

Plasmid	PTPase activity, units per mg of extract	
	Raytide	Angiotensin II
pKKUC12	0.3	<0.1
pKKUC-LCA	8280	1910
pKKUC-LAR	576	182
pKKUC-DLAR	44.3	4.2
pKKUC-DPTP	48.0	3.0
pKKUC-LCA 737S	4110	1530
pKKUC-LCA 828S	0.2	<0.1
pKKUC-LCA 1047S	8620	3410
pKKUC-LCA 1144S	5690	2230
pKKUC-LCA 737S 828S	0.2	<0.1
pKKUC-LCA 737S 1047S	5140	525
pKKUC-LCA 828S 1144S	0.2	<0.1
pKKUC-LCA 1047S 1144S	5170	964

Assays were done at 37°C for 30 min. Results in most cases are the average of two independent experiments.

Speculation. From the structure and enzymatic activity, there emerges a possible scenario for the function of these receptor-linked PTPases. When a proliferating cell that expresses, for example, DLAR, encounters another cell that expresses the cell-surface ligand for DLAR, the molecular interaction between these molecules may activate the cytoplasmic PTPase, causing cessation of cell growth by counteracting the effects of PTKases. This might be a mechanism that underlies the contact inhibition of cell growth and motility. It is even possible that the ligand of DLAR is another receptor-linked PTPase, such as DPTP, so that their effects are reciprocal. Because failure of this mechanism could result in unrestricted cell proliferation, these receptor-linked PTPases are candidates for anti-oncogenes (28).

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