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Molecular Cloning and Characterization of a Novel Human Receptor Protein Tyrosine Phosphatase Gene, *hPTP-J:* Down-Regulation of Gene Expression by PMA and Calcium Ionophore in Jurkat T Lymphoma Cells[1]

Bing Wang, Kenji Kishihara,[2] Donglei Zhang, Hiromitsu Hara, and Kikuo Nomoto
Department of Immunology, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-82 Japan

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A novel cDNA encoding a 1436 aa protein was cloned using a PCR system with degenerate primers. This new gene, *hPTP-J,* was found to encode a PTP protein consisting of an extracellular region containing an MAM (meprin, A5, μ)-like domain, an immunoglobulin-like domain, four fibronectin type-III repeats, a transmembrane region, and a cytoplasmic region containing two tandemly repeated PTP domains. hPTP-J is thus considered to be a new member of the type II receptor PTP (RPTP) subfamily, like RPTPμ and RPTPκ. *hPTP-J* gene expression was strongly detected in skeletal muscle and moderately detected in the prostate, pancreas, placenta, and heart, but was only weakly detected in the peripheral blood lymphocytes, thymus, and spleen even though gene expression was relatively high in the Jurkat T lymphoma cell line. Moreover, *hPTP-J* gene expression was down-regulated after Jurkat cells were stimulated by either PMA or calcium ionophore. Based on these findings, it is suggested that some signaling pathways mediated by PMA and/or intracellular calcium are involved in the regulation of *hPTP-J* gene expression in Jurkat cells.

It has been well established that the reversible phosphorylation of tyrosine residues plays a crucial regulatory role in various cellular events including cell differentiation, activation and proliferation (1). Thus, protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) are thought to be significantly involved in such events. Like PTKs, PTPs can be classified to two forms consisting of a receptor and a non-receptor form. The receptor PTPs (RPTPs) show a remarkable diversity in the extracellular domains (2). Based on this diversity, RPTPs have been classified into five subtypes (1-5). One of the most studied RPTPs, CD45, has a large extracellular domain that appears to be unrelated to that of any other RPTPs (6,7). We previously demonstrated the importance of CD45 in the development and function of T lymphocytes by gene targeting but the role of the extracellular domain remains controversial (8-11). The extracellular domains of other subclasses show similarities to immunoglobulin (Ig), MAM (meprin, A5, μ) or the fibronectin type III (FN III) domain and so on. For example, DEP1 has only 8 tandem FN III-like repeats (12), LAR contains 8 FN III and 3 Ig-like domains (13), PTPμ and PTPκ consist of 4 FN type III-like repeats, a single Ig-like domain and a newly identified MAM-like domain (14-16). The type II RPTP subfamily, including PTPκ and PTPμ, mediate the homophilic intercellular interactions (17,18) and the homophilic interaction depends on both MAM- and Ig-like domains (19,20). PTPκ and PTPμ can not interact with each other, which therefore suggests that they possess high specificities for homophilic binding (19).

In this manuscript, we described the putative human RPTP gene, *hPTP-J,* to be a new member of the type II RPTP subfamily. Interestingly, the *hPTP-J* gene expression was down-regulated by the stimulation of T lymphoma Jurkat cells with PMA or calcium ionophore and, as a result, the gene expression therefore appears to be regulated by such signal transduction pathways.

MATERIALS AND METHODS

Polymerase chain reaction (PCR) cloning of the putative PTP gene fragments. The sequences of the degenerate primers are as follows: sense primer: 5′-AITTCTGGIIIATGIIITGGGA-3′ (corresponding to "FWXMXW"), antisense primer: 5′-CT(G)ICCIIIICCIGCA(G)C-

[1] The hPTP-J nuclotide and deduced amino acid sequences reported in this article have been submitted to GenBank under Accession No. U73727.

[2] To whom correspondence should be addressed: Department of Immunology, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-82, Japan. Fax: +81-92-641-1315. E-mail: kishihar@bioreg.kyushu-u.ac.jp.

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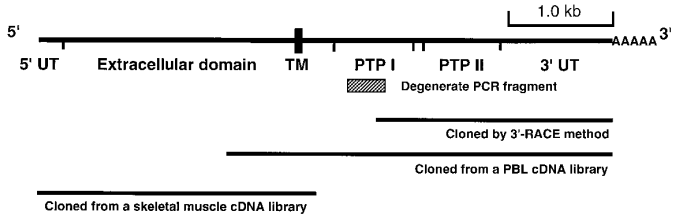

FIG. 1. Molecular cloning of *hPTP-J* cDNAs. Representative clones covered over the full length cDNA of the *hPTP-J* gene are shown. 5′UT, 5′-untranslated region; TM, transmembrane segment; PTP I, PTP domain I; PTP II, PTP domain II; 3′UT, 3′-untranslated region.

TA(G)CAA(G)TG-3′ (corresponding to "HCSAGXG"). Using Jurkat T lymphoma cDNA as a template, the PCR was performed as follows: the first 39 cycles: at 94°C for 1 min, at 37°C for 1 min and at 72°C for 1 min, the final cycle step: at 94°C for 1 min, at 37°C for 1 min and at 72°C for 4 min. The resulting RT-PCR fragments (*ca.* 350 bp) were subcloned into the pGEM-T vector (Promega). As a result of a sequencing and homology search using the BLAST in GenemeNet WWW server, one of the subclones was thus considered to be a part of a novel PTP gene.

3′-RACE cloning. To clone the cDNAs containing a 3′-untranslated region, 3′-RACE cloning was carried out. According to the sequence of the PCR fragment (*ca.* 350 bp), three gene-specific primers (GSP) were designed as follows: GSP1: 5′-CAAGCTGGTCGAGGTGAAAT-3′, GSP2: AGGACTCAGACACCTACG-3′, GSP3: AGTTCCACTTCACAGCGTGGC-3′. From the Jurkat cell-derived total RNA, cDNA was synthesized by the oligo(dT)-containing Adapter Primer (AP): 5′-GGCCACGCGTCGACTAGTAC$(T)_{17}$-3′. A 2.3kb fragment containing poly(A) was obtained by PCR with GSP and AP primers according to the manufacturer's instructions (3′-RACE System, LIFE TECHNOLOGIES).

Molecular cloning and sequencing of novel PTP cDNA clones. The 2.3 kb DNA fragment obtained by the 3′-RACE method was used as a probe for screening an oligo(dT)-primed cDNA library from human peripheral blood lymphocytes (PBL). A 3.5 kb cDNA fragment containing a poly(A) tail was obtained. The 5′-part of the *hPTP-J* gene was obtained by screening a skeletal muscle cDNA library (oligo(dT) plus random hexamer-primed and 5′-stretched, CLONTECH). The obtained cDNA clones were subcloned and sequenced by the dideoxy chain-termination method using the PRISM Dye Deoxy Terminator Cycle Sequencing Kit and Model 377 DNA sequencer (Perkin Elmer). The final sequence was confirmed from both strands. The nucleotide sequences from the cDNA libraries and Jurkat were identical. A homology search of the *hPTP-J* gene with known PTP genes was also performed by BLAST.

RNA isolation and Northern blot analysis. Total RNAs were extracted from human tumor cell lines: HeLa (epitheloid carcinoma), Jurkat (acute T cell leukemia), PC-9 (lung carcinoma), A549 (lung carcinoma) and PBL by either the guanidine isothiocyanate/acid-phenol method (21) or by the QuickPrep Total RNA Extraction Kit (Pharmacia Biotech). Ten to twenty μg total RNA of each sample were electrophoresed on a 0.8% agarose gel containing formamide and then were transferred to a nylon membrane (Hybond-N, Amersham). To analyze the tissue distribution of the *hPTP-J* gene expression, Northern blot membranes with mRNAs from various human tissue specimens (Multiple Tissue Northern blots, CLONTECH) were hybridized according to the manufacturer's instructions. A radio-labeled DNA fragment of the *hPTP-J* domain I, the extracellular portion containing FN III or β-actin was used as a probe for Northern hybridization.

Stimulation of PBL and Jurkat cells. PBL and Jurkat cells were cultured in a complete RPMI-1640 medium (LIFE TECHNOLOGIES) supplemented with 10% fetal bovine serum (INTERGEN). Phorbol 12-myristate 13-acetate (PMA, 50 ng/ml, Sigma), phytohemagglutinin-P (PHA, 5 μg/ml, Sigma), calcium ionophore A23187 (Ca, 200 ng/ml or 500 ng/ml, Sigma) or recombinant human IL-2 (rhIL-2, 50 U/ml, Takeda Chemical Industries) was added to the culture as indicated in the legend of Fig.5.

RESULTS AND DISCUSSION

Cloning and structural characterization of hPTP-J cDNA. One of approximately 350 bp fragments encoding a part of a putative novel PTP domain was obtained by the PCR system with degenerate primers. Several cDNA clones covering the full length of the novel PTP gene were cloned from PBL and skeletal muscle cDNA libraries (Fig.1). As a result of such sequencing, *hPTP-J* was 5,589 bp with a 4,308 bp open reading frame encoding a 1436 amino acid polypeptide. The nucleotide sequence of the full length *hPTP-J* gene and its deduced amino acid sequence are shown in Fig.2. The proposed initiation codon (ATG) was determined according to the Kozak rules because the initiation site is flanked by a guanine-rich sequence (22). The initiating methionine is followed by a hydrophobic sequence that may serve as a signal sequence (18 aa). A second hydrophobic region spanned from aa 745 to 770 as a transmembrane segment (26 aa), followed by two tandemly repeated PTP domains in the cytoplasmic region. It can thus be predicted that the novel PTP protein contains the putative mature extracellular part of 726 aa and the cytoplamic part of 666 aa as shown in Fig.2. There are 9 putative *N*-glycosylation sites in the extracellular region. Interestingly, the extracellular region is composed of an MAM-like domain, an Ig-like domain and 4 FN III repeats from the *N*-terminus, thus suggesting hPTP-J to be a new member of type II RPTPs including PTPκ and PTPμ. According to a homology search of amino acid sequences between hPTP-J and human PTPκ/PTPμ, the homologies were significantly higher in the PTP I (hPTPκ/hPTP-J: 81.4%, hPTPμ/hPTP-J: 82.4 %) and PTP II (79.7%, 78.4%) domains than in their MAM-like (66.1%, 67.3%) and Ig-like (69.6%, 46.4%) domains which, as a result, suggested a differ-

FIG. 2. Nucleotide and deduced amino acid sequences of *hPTP-J* cDNA. The amino acid sequences are shown in one-letter code. The translation stop codon is indicated by an asterisk. The putative signal sequence is underlined with a thick line at the top of the coding region. The putative glycosylation sites (N-X-S/T) are underlined with a thin line. The putative transmembarane segment is indicated by an open box. The putative PTP I and II domains are shown on shaded backgrounds. The polyadenylation signal sequence is underlined at the 3′UT. The nucleotide sequences complementary to the degenerate PCR primers in the PTP domain I are indicated by boldface letters.

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cagtcccgctccgcgccgcgccgctccgctccggctcgggctccggctcgcctcgggctgggctcgggctccgggggcggcgtcccgcgccgggccccgggacgcggcgatcgtccaacc nt-120 ~  -1
ATGGCCCGTGCCCAGGCGCTCGTGCTGGCACTCACCTTCCAGCTCTGCGCGCCGGAGACCGAGACTCCGGCAGCTGGCTGCACCTTCGAGGAGGCAAGTGACCCAGCAGTGCCCTGCGAG nt   1 ~  120
 M  A  R  A  Q  A  L  V  L  A  L  T  F  Q  L  C  A  P  E  T  E  T  P  A  A  G  C  T  F  E  E  A  S  D  P  A  V  P  C  E  aa   1 ~   40
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 Y  S  Q  A  Q  Y  D  D  F  Q  W  E  Q  V  R  I  H  P  G  T  R  A  P  A  D  L  P  H  G  S  Y  L  M  V  N  T  S  Q  H  A  aa  41 ~   80
CCAGGCCAGCGAGCCCATGTCATCTTCCAGAGCCTGAGCGAGAATGATACCCACTGTGTGCAGTTCAGCTACTTCCTGTACAGCCGGGACGGGCACAGCCCGGGCACCCTGGGCGTCTAC nt 241 ~  360
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GTGCGCGTTAATGGGGGCCCCTGGGCAGTGCTGTGTGGAATATGACTGGATCCCACGGCCGTCAGTGGCACCAGGCTGAGCTGGCTGTCAGCACTTTCTGGCCCAATGAATATCAGGTG nt 361 ~  480
 V  R  V  N  G  G  P  L  G  S  A  V  W  N  M  T  G  S  H  G  R  Q  W  H  Q  A  E  L  A  V  S  T  F  W  P  N  E  Y  Q  V  aa 121 ~  160
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 N  A  G  Q  N  A  S  F  Q  C  M  A  A  G  R  A  A  E  A  E  R  F  L  L  Q  R  Q  S  G  A  L  V  P  A  A  G  V  R  H  I  aa 201 ~  240
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 S  H  R  R  F  L  A  T  F  P  L  A  A  V  S  R  A  E  Q  D  L  Y  R  C  V  S  Q  A  P  R  G  A  G  V  S  N  F  A  E  L  aa 241 ~  280
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 I  V  K  E  P  P  T  P  I  A  P  P  Q  L  L  R  A  G  P  T  Y  L  I  I  Q  L  N  T  N  S  I  I  G  D  G  P  I  V  R  K  aa 281 ~  320
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ACGGATGAGGATGTGCCCAGTGGGATTGCAGCCGAGTCCCTGACCTTCACTCCACTGGAGGACATGATCTTCCTCAAGTGGGAGGAGCCCCAGGAGCCCAATGGTCTCATCACCCAGTAT nt1441 ~ 1560
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GAGATCAGCTACCAGAGCATCGAGTCATCAGACCCGGCAGTGAACGTGCCAGGCCCACGACGTACCATCTCCAAGCTCCGCAATGAGACCTACCATGTCTTCTCCAACCTGCACCCAGGC nt1561 ~ 1680
 E  I  S  Y  Q  S  I  E  S  S  D  P  A  V  N  V  P  G  P  R  R  T  I  S  K  L  R  N  E  T  Y  H  V  F  S  N  L  H  P  G  aa 521 ~  560
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 L  G  E  S  E  N  T  I  T  V  L  L  R  P  A  Q  G  R  G  A  P  I  S  V  Y  Q  V  I  V  E  E  E  R  A  R  R  L  R  R  E  aa 601 ~  640
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 D  I  K  I  M  L  V  K  T  E  T  L  A  E  Y  V  V  R  T  F  A  L  E  R  R  G  Y  S  A  R  H  E  V  R  Q  F  H  F  T  A  aa1001 ~ 1040
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GACGTCCTGCCGCCCGACCGCTGCCTGCCCTTCCTCATCTCCACTGATGGGGACTCCAACAACTACATTAATGCAGCCCTGACTGACAGCTACACACGGAGTGCGGCCTTCATCGTGACC nt3601 ~ 3720
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 Y  W  P  E  P  G  R  Q  Q  Y  G  L  M  E  V  E  F  M  S  G  T  A  D  E  D  L  V  A  R  V  F  R  V  Q  N  I  S  R  L  Q  aa1281 ~ 1320
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 E  G  H  L  L  V  R  H  F  Q  F  L  R  W  S  A  Y  R  D  T  P  D  S  K  K  A  F  L  H  L  L  A  E  V  D  K  W  Q  A  E  aa1321 ~ 1360
AGTGGGGATGGGCGCACCATCGTGCACTGCCTAAACGGGGGAGGACGCAGCGGCACCTTCTGCGCCTGCGCCACGGTCCTGGAGATGATCCGCTGCCACAACTTGGTGGACGTTTTCTTT nt4081 ~ 4200
 S  G  D  G  R  T  I  V  H  C  L  N  G  G  G  R  S  G  T  F  C  A  C  A  T  V  L  E  M  I  R  C  H  N  L  V  D  V  F  F  aa1361 ~ 1400
GCTGCCAAAACCCTCCGGAACTACAAACCCAACATGGTGGAGACCATGGATCAGTACCACTTTGCTACGATGTGGCCCTGGAGTACTTGGAGGGGCTGGAGTCAAGATAGCggggccct nt4201 ~ 4320
 A  A  K  T  L  R  N  Y  K  P  N  M  V  E  T  M  D  Q  Y  H  F  C  Y  D  V  A  L  E  Y  L  E  G  L  E  S  R  *            aa1401 ~ 1436
ggcctggggcacccactgcacactcagggccagacccaccatcctggactggcgaggaagatcagtgcctcctgctctgcccaaacacactcccatggggcaagcactggagtggatgct nt4321 ~ 4440
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ggccctgcagagagcatcccaggccaaggttcccactcagcctgccccctctgcatgtgggtagaggatgtactggggacttggcatttaggattccatctggcccagccccctgaaggtcc nt4681 ~ 4800
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agctgatgtttgtaaagcgctttgtaaataaacgtgctctctgaatgccaaaaaaaaaaaaaaaaaaa nt5401 ~ 5469
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ent ligand specificity for the extracellular domain between hPTP-J and PTPκ or PTPμ.

Using a procaryotic expression system, a GST-fusion protein including the cytoplasmic region of hPTP-J was prepared and their PTP activities were assayed using p-nitrophenol phosphate as a substrate according to the method of Frangioni *et al* (23). Their PTP activities were undetectable as observed in PTP-mIA-2 (data not shown), thus implying hPTP-J to either have a strict substrate specificity or that the procaryotic expression system is simply not suitable for producing active hPTP-J (24).

Expression of hPTP-J gene in various tissues specimens and cell lines. The tissue distribution of the *hPTP-J* gene expression was examined by a Northern blot analysis. The expression of *hPTP-J* mRNA (ca. 5.6 kb in length) detected by the probe of the PTP domain I was limited to skeletal muscle, heart, prostate, pancreas and placenta but was only slightly detected in the thymus, spleen and PBL (Fig.3a). Using the extracellular domain probe including a part of the FN III repeats, a 2.6 kb mRNA band was also observed in addition to the *hPTP-J* band in skeletal muscle, heart, prostate and small intestine, and an additional 1.0 kb band was also predominantly observed in small intestine (Fig.3b). these results imply the presence of genes encoding a domain highly homologous to the extracellular domain of *hPTP-J*. The gene expression of *hPTP-J* in several human cell lines was observed. As shown in Fig.4, the *hPTP-J* gene expression was markedly high in Jurkat T lymphoma compared with that in PC-9 (lung carcinoma), HeLa (epitheloid carcinoma) and A549 (lung carcinoma). Since no *hPTP-J* gene expression was detectable in the PBL or spleen, oncogenesis may be associated with the *hPTP-J* gene expression in Jurkat T lymphocytes.

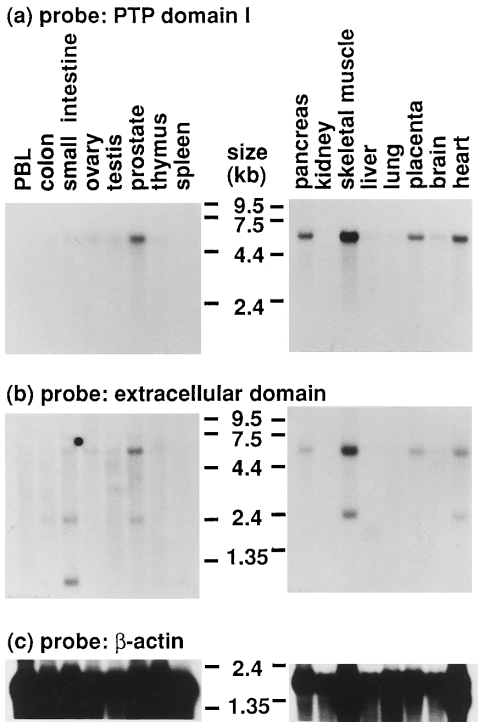

FIG. 3. A Northern blot analysis of the *hPTP-J* gene expression in various tissues and organs. The membranes were blotted with 2 μg each of high-quality poly(A)$^+$ mRNA isolated from the indicated human tissues and organs. Hybridization was done using the [^{32}P]-labeled DNA fragment of the PTP domain I (a), the extracellular domain (b), and β-actin as control (c).

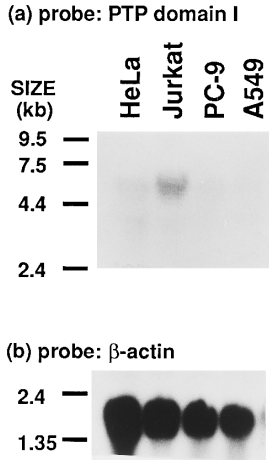

FIG. 4. A Northern blot analysis of the *hPTP-J* gene expression in tumor cell lines. Twenty μg each of total RNA prepared from human tumor cell lines, HeLa, Jurkat, PC-9 and A549 were blotted on a nylon membrane. Hybridization was done using the [^{32}P]-labeled DNA fragment of the PTP domain I (a) and β-actin as a control (b).

Down-regulation of hPTP-J gene expression in Jurkat cells by stimulation with PMA and calcium ionophore. In order to analyze the regulation of the *hPTP-J* gene expression, PBL and Jurkat cells were examined under various stimulation conditions. First, the *hPTP-J* gene expression was detected neither in non-stimulated PBL nor in PBL stimulated with PHA or IL-2 (Fig.5). On the other hand, a relatively high level of *hPTP-J* mRNA was observed in Jurkat cells and the level did not changed after the stimulation with PHA or IL-2 (Fig.5). Surprisingly, *hPTP-J* mRNA diminished in the Jurkat cells stimulated with only PMA (Fig.5). As reported previously, a longer exposure (more than 24 hr) of Jurkat cells to high concentrations of PMA leads to the down-regulation of PMA-sensitive PKC isoforms, PKC-α and -βI (25), thus indicating that the disappearence of the *hPTP-J* gene expression in Jurkat cells by PMA may be associated with the PMA-induced depletion mechanism of PKC isoforms. Interestingly, the *hPTP-J* level was also down-regulated in Jurkat cells by stimulating with A-23187 (Fig.5). These findings therefore suggest that the regulation mechanism of the *hPTP-J* gene expression appears to be

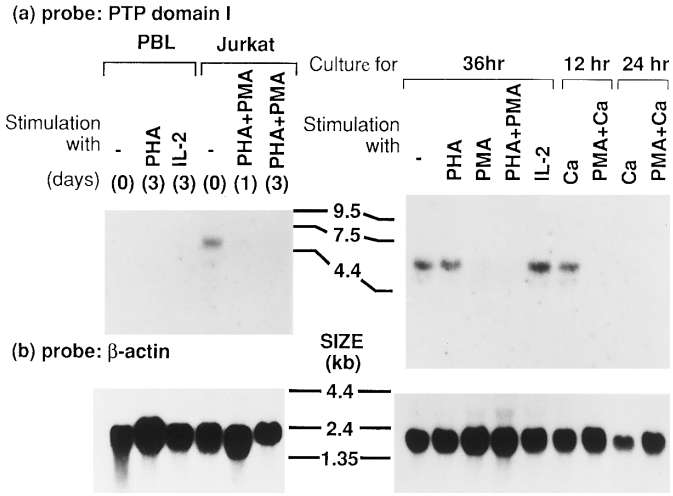

FIG. 5. A Northern blot analysis of *hPTP-J* gene expression in peripheral blood lymphocytes and Jurkat cells. Twenty μg each of total RNA prepared from human peripheral blood lymphocytes (PBL) and Jurkat cells were blotted on a nylon membrane. PBL and Jurkat cells were cultured at various conditions as indicated both in the figure and under Materials and Methods. To stimulate the cells, PMA (50 ng/ml), PHA (5 μg/ml), Ca (A-23187, 200 ng/ml or 500 ng/ml) or rhIL-2 (50 U/ml) and their combinations were added to the culture media as indicated in the figure. Hybridization was done using the [^{32}P]-labeled DNA fragment of the PTP domain I (a) and β-actin as a control (b). 10 μg of total RNA was loaded only in the sample of the Jurkat cells cultured with Ca for 24 hr.

closely associated with either the PMA or intracellular calcium mediated signaling pathways.

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