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Cloning, heterologous expression, and chromosomal localization of human inositol polyphosphate 1-phosphatase

(inositol phosphate/lithium)

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ABSTRACT **Inositol polyphosphate 1-phosphatase, an enzyme in the phosphatidylinositol signaling pathway, catalyzes the hydrolysis of the 1 position phosphate from inositol 1,3,4-trisphosphate and inositol 1,4-bisphosphate. We used a cDNA that encodes bovine inositol polyphosphate 1-phosphatase as a probe to isolate the human counterpart by low-stringency hybridization. The 1.74-kb human cDNA has 341 bp of 5′ untranslated region, 180 bp of 3′ untranslated region, poly(A)$_{32}$, and predicts a protein of 399 amino acids. Human and bovine inositol polyphosphate 1-phosphatases show 84% amino acid sequence identity. Northern blot analysis from a variety of human tissues demonstrates that a 1.9-kb mRNA is ubiquitously expressed with highest levels in pancreas and kidney. Several higher molecular weight mRNAs also are expressed in brain, muscle, heart, and liver. We have confirmed the functional identity of the human cDNA by heterologous expression in NIH 3T3 fibroblasts, COS-7 cells and *Escherichia coli*. Polymerase chain reaction assay of a panel of human–rodent somatic cell hybrid DNA using human inositol polyphosphate 1-phosphatase-specific DNA primers resulted in amplification of a specific product using chromosome 2 DNA as template. Fluorescence *in situ* hybridization of metaphase chromosomes localizes the gene to chromosome 2 band q32. The identification of the human inositol polyphosphate 1-phosphatase gene locus provides a target for linkage analysis to identify defects in patients with inherited psychiatric disorders that respond to lithium ions, an inhibitor of the enzyme.**

Cells respond to extracellular stimuli through complicated networks of responses. Phosphatidylinositol turnover plays a key role in intracellular signaling (1–4). Agonist-induced stimulation of cells releases the signaling molecules diacylglycerol and inositol polyphosphates via phospholipase C hydrolysis of phosphatidylinositols. Diacylglycerol functions to stimulate protein kinase C (5), and several inositol polyphosphates, most notably inositol 1,4,5-trisphosphate, evoke the release of intracellular and intercellular Ca^{2+} (6). Action of inositol phosphate phosphatases and kinases gives rise to a plethora of inositol polyphosphates (7) in the cytosol that may also serve as signaling or regulatory molecules. Inositol 1,4-bisphosphate [Ins(1,4)P_2] and inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3] are metabolites whose functions remain unknown. Reports that Ins(1,4)P_2 stimulates the low activity form of DNA polymerase α (8) and Ins(1,3,4)P_3 mobilizes Ca^{2+} (9, 10) suggest possible functions for these inositol phosphates.

Ins(1,3,4)P_3 is a pivotal metabolite that is the first compound in three pathways. Ins(1,3,4)P_3 is phosphorylated by Ins(1,3,4)P_3 6-kinase (11–14) to form Ins(1,3,4,6)P_4, which is the precursor for formation of other inositol polyphosphates, including InsP_5, InsP_6, and other InsP_4 isomers (11–18). Inositol polyphosphate 1-phosphatase hydrolyzes the 1 position phosphate of both Ins(1,4)P_2 and Ins(1,3,4)P_3 (19–22). Ins(1,3,4)P_3 is also metabolized by inositol polyphosphate 4-phosphatase (23, 24) to form Ins(1,3)P_2.

Inositol polyphosphate 1-phosphatase is one of two enzymes involved in inositol phosphate metabolism that is uncompetitively inhibited by lithium ions (20–22), the other being inositol monophosphate phosphatase (25). Patients with psychiatric disorders treated with lithium ions achieve intracellular lithium levels sufficient to inhibit inositol polyphosphate 1-phosphatase hydrolysis of Ins(1,3,4)P_3 (K_i = 0.3 mM) (22, 26, 27).

We report the cloning and chromosomal localization of human inositol polyphosphate 1-phosphatase. Identification of the gene encoding human inositol polyphosphate 1-phosphatase provides a target for linkage analysis to identify defects in patients with inherited psychiatric disorders.‡

MATERIALS AND METHODS

Materials. A λgt11 human umbilical vein endothelial cell cDNA library (28) was provided by J. Evan Sadler (Washington University). *Escherichia coli* strains Y1088 and XL1-Blue, Epicurian Coli XL1-Blue competent cells, Bluescript SK+ II plasmid, and Lambda FIX human lung fibroblast genomic library were purchased from Stratagene. Nitrocellulose filters were purchased from Schleicher & Schuell. [α-^{32}P]dATP, [γ-^{32}P]ATP, and deoxyadenosine 5′ [[α-^{35}S]thio]triphosphate were purchased from Amersham. PCR reagents were from Perkin–Elmer. A Sequenase sequencing kit was purchased from United States Biochemical. Restriction enzymes, G-25 Sephadex spin columns, random hexamer radiolabeling kit, yeast tRNA, and salmon sperm DNA were purchased from Boehringer Mannheim. Human multiple tissue Northern blot was purchased from Clontech. Oligonucleotides were synthesized on an Applied Biosystems model 380B DNA synthesizer. Mammalian expression plasmid pCDNeo was purchased from Invitrogen (San Diego). Lipofectin was purchased from Bethesda Research Laboratories. Fluorescein-conjugated avidin distinct cell sorting and fluorescein-conjugated goat anti-avidin D antibody were purchased from Vector Laboratories. Other reagents were from Sigma.

Human cDNA Cloning. Fifty nanograms of a 1.6-kb DNA fragment encoding bovine inositol polyphosphate 1-phosphatase (29) were radiolabeled with random hexamer primers and [α-^{32}P]dATP (specific activity of probe was 2.4 × 10^9 cpm per μg of DNA). This fragment was used to screen duplicate filters representing 1 × 10^6 recombinant clones from a λgt11 human umbilical vein endothelial cell library using low-

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Abbreviations: Ins(1,4)P_2, inositol 1,4-bisphosphate; Ins(1,3,4)P_3, inositol 1,3,4-trisphosphate.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. L08488).

stringency hybridization and washing conditions. Hybridization was performed for 14 hr at 37°C in 40% formamide/6× standard saline citrate (SSC)/5× Denhardt's solution/0.1 mg of sheared salmon sperm DNA per ml/0.1% SDS. Filters were washed at room temperature with 3× SSC/0.1% SDS followed by 1× SSC/0.1% SDS and exposed to film. A single positive clone, λ5-1, was isolated, λ phage DNA was prepared, and the 1.2-kb *Eco*RI insert was subcloned into Bluescript SK+ II (30). Sequencing was performed by dideoxynucleotide chain termination (31) with Sequenase version 2.0 according to the manufacturer's instructions (32). Subsequent clones were identified by rescreening the same library with the radiolabeled 1.2-kb *Eco*RI insert from clone λ5-1. Five positive clones were isolated and the inserts were subcloned as described above. The 1.74-kb insert of clone λ7-1 was sequenced in its entirety on both strands.

Northern Blot Analysis. Thirty nanograms of a 1.74-kb human inositol polyphosphate 1-phosphatase cDNA fragment was radiolabeled as described above (specific activity of probe was 1.9×10^9 cpm per μg of DNA) and used to probe a human multiple tissue Northern blot purchased from Clontech. This blot was prepared with 2 μg of poly(A) selected mRNA isolated from various human tissues. The membrane was prehybridized and hybridized at 42°C in 10 ml of 50% formamide/5× SSPE (1× SSPE = 0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA)/10× Denhardt's solution/0.1 mg of sheared salmon sperm DNA per ml/2% SDS. The membrane was washed in 0.1× SSC/0.1% SDS at room temperature and exposed to film. Subsequent high-stringency wash was performed in 0.1× SSC/0.1% SDS at 45°C and the blot was reexposed to film. The same blot was reprobed with human β-actin DNA as described for human inositol polyphosphate 1-phosphatase to control for mRNA amounts loaded.

Expressional Studies of Human Inositol Polyphosphate 1-Phosphatase. The mammalian expression plasmid, pCDNeoH1pt, was made by inserting the 1.2-kb *Eco*RV/*Pst* I DNA fragment representing human inositol polyphosphate 1-phosphatase coding sequence at the *Hin*dIII and *Bam*HI linker sites of pCDNeo using 5′ adapter oligonucleotides 5′-AGCTTGAATTCGCTAGCATGTCAGAT-3′ and 5′-ATCTGACATGCTAGCGAATTCA-3′ and 3′ adapter oligonucleotides 5′-GAGACGCATACCTAGCTAGCTC-GAGAATTCG-3′ and 5′-GATCCGAATTCTC-GAGCTAGCTAGGTATGCGTCTCTGCA-3′. Five micrograms of CsCl-purified pCDNeoH1pt or pCDNeo control was transfected into 3×10^5 NIH 3T3 fibroblast cells using the cationic lipid Lipofectin according to the manufacturer's instructions. Forty-eight hours after transfection, cells were treated with trypsin, diluted 1:5, and selected with 500 μg of G418 per ml for 14 days. Polyclonal populations of G418-resistant cells, representing >25 individual colonies, were amplified and used to prepare cell extracts as follows: 80% confluent cells were scraped from plates, pelleted, resuspended in sonication buffer (20 mM Hepes, pH 7.5/3 mM $MgCl_2$/0.5 mM phenylmethylsulfonyl fluoride), and sonicated on ice for 15 sec two times. Cellular debris was pelleted at 16,000 × *g* for 10 min and supernatants were tested for inositol polyphosphate 1-phosphatase activity using [^{3}H]-Ins(1,4)P_2 as described (20).

Transient transfections were performed in 50% confluent COS-7 cells using 50 μg of Lipofectin and 20 μg of either pCDNeo or pCDNeoH1pt plasmids for 12 hr, at which time the Lipofectin/DNA was removed and the cells were incubated for 48 hr in medium containing 10% fetal calf serum. Cells were harvested, and cell extracts were prepared and assayed as described for stably transfected cells.

Bacterial expression plasmid pTrpH1pt was made by cutting the cDNA fragment containing the *Bam*HI linker described above with *Hin*dIII and, using the 5′ adapter oligonucleotides 5′-TATGTCAGAT-3′ and 5′-ATCTGAC-3′, ligating it into the *Nde* I and *Bam*HI sites of pTrp (27, 30). Extracts from logarithmic phase *E. coli* strain XL1-Blue harboring pTrpH1pt or pTrp plasmids were made and assayed as described above.

PCR of Somatic Cell Hybrid DNA. Sense 5′-GTCAC-CATTTTAATTGGTGTC-3′ and antisense 5′-CACCT-GAGGGTGTTTGGATCT-3′ oligonucleotides corresponding to amino acids 200–207 and 226–233, respectively, were used as primers to amplify a 100-bp PCR product from a panel of human–rodent somatic cell hybrid DNA [National Institute of General Medical Sciences (NIGMS) Human Genetic Mutant Cell Repository, Camden, NJ; kindly provided by Matthew Holt, Washington University]. PCRs were performed in 25 μl as described (33) for 30 cycles with 25 ng of template DNA and 150 ng of sense and antisense primers with each cycle consisting of denaturation (94°C), annealing (55°C), and elongation (72°C) steps of 1 min each. Reaction products were subjected to 2.5% agarose/TBE electrophoresis (30), stained with ethidium bromide, and visualized under ultraviolet light.

Isolation of Human Genomic Clone. Twenty nanograms of the gel-purified 100-bp PCR product amplified from human inositol polyphosphate 1-phosphatase cDNA template as described above was end-labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP (specific activity of probe was 1.9×10^8 cpm/μg) and used to screen 7×10^5 recombinants from a Lambda FIX human lung fibroblast genomic DNA library. Duplicate filters were prehybridized at 50°C for 1 hr in 6× SSC/5× Denhardt's solution/0.05% sodium pyrophosphate/0.1 mg of sheared salmon sperm DNA per ml/0.5% SDS and hybridized with labeled probe at 60°C for 15 hr in 6× SSC/5× Denhardt's solution/0.05% sodium pyrophosphate/0.1 mg of yeast tRNA per ml. Filters were washed at 60°C in 6× SSC/0.05% sodium pyrophosphate and exposed to film at −70°C with intensifying screens for 20 hr. Positive clones were isolated and λ phage DNA was prepared. The insert of the genomic clone λG3-1 was analyzed by PCR as described for somatic cell DNA hybrid PCR and also by sequencing with inositol polyphosphate 1-phosphatase-specific primers located throughout the cDNA.

Gene Mapping by *in Situ* Hybridization. Fluorescence *in situ* hybridization was performed as described (34). Human prometaphase chromosome spreads were prepared from cultured phytohemagglutinin-stimulated peripheral blood lymphocytes from a male with a normal karyotype (46XY). Extended chromosomes were produced by colchicine treatment (35). One hundred and fifty nanograms of a 12.2-kb human inositol polyphosphate 1-phosphatase genomic clone, λG3-1, was labeled with biotin-11-dUTP by nick-translation (36) and hybridized to human chromosomal spreads (35). For fluorochrome detection, slides were incubated with fluorescein-conjugated avidin DCS at 5 μg/ml, amplified by incubation with fluorescein-conjugated goat anti-avidin D antibodies at 5 μg/ml, and counterstained with 4,6-diamidino-2-phenylindole dihydrochloride at 200 ng/ml and propidium iodide at 200 ng/ml, which were present in the final wash solution. Cytogenetic banding patterns were observed by staining the slides with Giemsa following fluorescent hybridization.

RESULTS

We have isolated a human inositol polyphosphate 1-phosphatase cDNA from human umbilical vein endothelial cells using low-stringency hybridization. The 1.6-kb bovine inositol polyphosphate 1-phosphatase cDNA (27) was initially used to identify a 1.2-kb partial human cDNA, λ5-1. This was used to rescreen the library yielding a 1741-bp clone, λ7-1, that encodes a protein of 399 amino acids and has 341 bp of 5′ untranslated sequence, 180 bp of 3′ untranslated region,

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1   MSDILQELLRVSEKAANIARACRQQETLFQLLIEEKKEGEKNKKFAVDFK  50   Bovine
    |||||.|||  ||||||||||||||||||.||||||||||||||||||||
1   MSDILRELLCVSEKAANIARACRQQEALFQLLIEEKKEGEKNKKFAVDFK  50   Human

51  TLADVLVQEVIKENMENKFPGLGKKIFGEESNELTNDLGEKIIMRLGPTE  100  Bovine
    |||||||||||||:||||||||||:|.|||||||||:|||:||||.:||..||
51  TLADVLVQEVIKQNMENKFPGLEKNIFGEESNEFTNDWGEKITLRLCSTE  100  Human

101 EETVALLSKVLNGNKLASEALAKVVHQDVFFSDPALDSVEINIPQDILGI  150  Bovine
    |||..|||||||||||:||||||:|||||| |.||.|||.|||:|||||||
101 EETAELLSKVLNGNKVASEALARVVHQDVAFTDPTLDSTEINVPQDILGI  150  Human

151 WVDPIDSTYQYIKGSADITPNQGIFPSGLQCVTVLIGVYDIQTGVPLMGV  200  Bovine
    ||||||||||||||||||..||||||:|||||||:||||||||||||||||
151 WVDPIDSTYQYIKGSADIKSNQGIFPCGLQCVTILIGVYDIQTGVPLMGV  200  Human

201 INQPFVSQDLHTRRWKGQCYWGLSYLGTNIHSLLPPVSTRSNSEAQSQGT  250  Bovine
    |||||||.|  :|  |||||||||||||:|||:|||   .:|  |..||.:   |
201 INQPFVSRDPNTLRWKGQCYWGLSYMGTNMHSLQLTISRRNGSETH...T  247  Human

251 QNPSSEG..SCRFSVVISTSEKETIKGALSHVCGERIFRAAGAGYKSLCV  298  Bovine
    .|.:||:   |..||.||||||||||:|||:|||:||| ||||||||||||
248 GNTGSEAAFSPSFSAVISTSEKETIKAALSRVCGDRIFGAAGAGYKSLCV  297  Human

299 ILGLADIYIFSEDTTFKWDSCAAHAILRAMGGGMVDLKECLERNPDTGLD  348  Bovine
    : ||.|||||||||||||||||||||||||||||||:||||||||||:||||
298 VQGLVDIYIFSEDTTFKWDSCAAHAILRAMGGGIVDLKECLERNPETGLD  347  Human

349 LPQLVYHVGNEGAAGVDQWANKGGLIAYRSEKQLETFLSRLLQHLAPVAT  398  Bovine
    |||||||:|||||||||.||||||||||| |.|||||| |:|:||||..|
348 LPQLVYHVENEGAAGVDRWANKGGLIAYRSRKRLETFLSLLVQNLAPAET  397  Human

399 HT 401                                               Bovine
    ||
398 HT 400                                               Human
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FIG. 1. Sequence alignment of bovine and human inositol polyphosphate 1-phosphatase. Primary sequences were aligned using the Genetics Computer Group program GAP (37). Vertical bar represents sequence identity, double dot indicates conserved residues, and single dot denotes single nucleotide change. Amino acids are given by single-letter code.

and an AAUAAA putative polyadenylylation signal located 16 nucleotides upstream of a poly(A)$_{32}$ tail (sequence not shown). The full-length mRNA based on Northern blot analysis is 1.9 kb (see below), suggesting that clone λ7-1 may be missing some 5′ untranslated region. Alignment of the human and bovine predicted amino acid sequence (Fig. 1) shows 84% identity. We found no other related sequences upon searching GenBank and EMBL data bases using on-line TBLASTN service (38) as of February 1993 except for motifs previously described (29, 39).

We have expressed the human cDNA in mammalian and bacterial cells to confirm that the isolated human clone encodes a functionally active inositol polyphosphate 1-phosphatase. The human inositol polyphosphate 1-phosphatase open reading frame was inserted into the mammalian expression plasmid pCDNeo, harboring a cytomegalovirus promoter and neomycin-resistance marker. NIH 3T3 fibroblast cells were transfected with pCDNeoH1pt or pCDNeo plasmids and subjected to G418 selection. Stably transfected cells were pooled; cytosolic extracts were prepared and assayed for inositol polyphosphate 1-phosphatase enzymatic activity. Cells harboring pCDNeoH1pt expressed 5-fold increased inositol polyphosphate 1-phosphatase enzyme activity relative to cells transfected with pCDNeo (Table 1). We also transiently transfected COS-7 monkey cells with either pCDNeo or pCDNeoH1pt and measured levels of enzyme activity. Cells transfected with pCDNeoH1pt expressed 10-fold greater levels of inositol polyphosphate 1-phosphatase than pCDNeo control transfected cells (Table 1). In addition, we inserted the coding region of the human cDNA into a tryptophan promoter/operator bacterial expression plasmid, yielding pTRPH1pt. Cellular extracts were prepared and assayed for enzyme activity from logarithmic growth phase cultures of *E. coli* strain XL1-Blue transformed with pTRPH1pt or control plasmid pTRP (Table 1). Overexpressing bacteria had inositol polyphosphate 1-phosphatase levels 20 times greater than in control cells. The properties of the recombinant human enzyme were similar to native bovine inositol polyphosphate 1-phosphatase including inhibition by lithium ions (data not shown).

Table 1. Heterologous expression of human inositol polyphosphate 1-phosphatase

Cell line	Specific activity, nmol·min^{-1}·mg^{-1}	Relative expression
pCDNeo/NIH 3T3	0.8	1
pCDNeoH1pt/NIH 3T3	4.3	5.4
pCDNeo/COS-7	1.0	1
pCDNeoH1pt/COS-7	10.2	10.2
pTRP/XL1-Blue	0.1	1
pTRPH1pt/XL1-Blue	2.0	20

Specific activity reflects the amount of Ins(1,4)P_2 hydrolyzed to Ins(4)P per min per mg of crude cellular protein. Relative expression indicates increase in inositol polyphosphate 1-phosphatase specific activity relative to control.

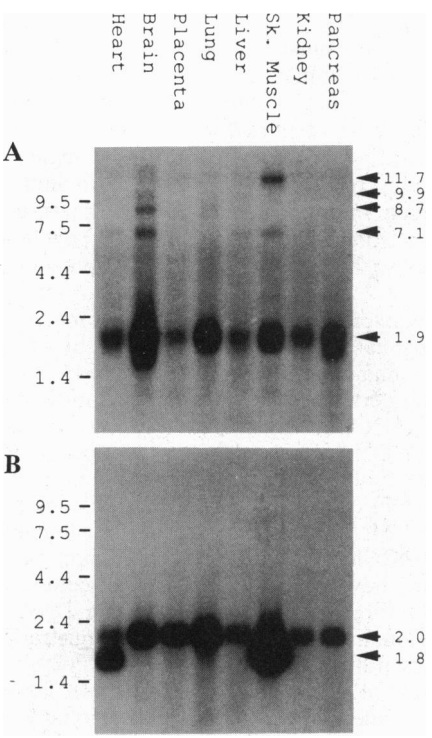

FIG. 2. Distribution of inositol polyphosphate 1-phosphatase mRNA in human tissues. (*A*) Two micrograms of polyadenylylated RNA was probed with radiolabeled human inositol polyphosphate 1-phosphatase cDNA and exposed to film for 44 hr at −70°C with an intensifying screen. (*B*) The blot was then stripped and reprobed with human β-actin cDNA and exposed to film for 1.5 hr at 25°C. RNA molecular size standards (kb) are shown on the left and calculated molecular sizes of individual species, denoted by arrows, are shown on the right.

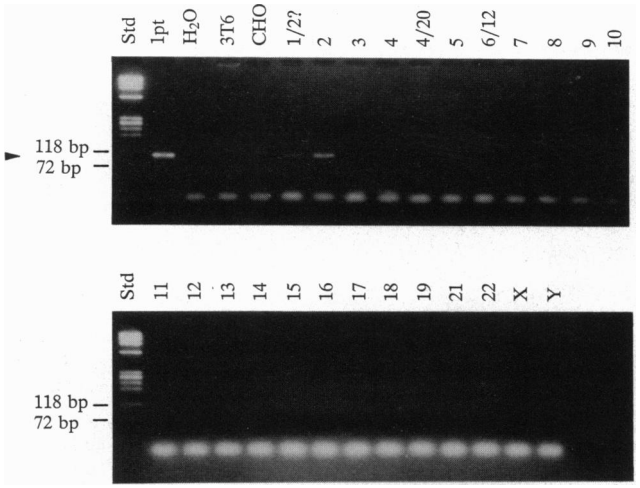

FIG. 3. PCR of a panel of somatic cell line hybrid DNAs using human inositol polyphosphate 1-phosphatase-specific primers. Sense and antisense primers directed to amino acids 200–207 and 226–233, respectively, of the human cDNA were used to amplify a 100-bp product (denoted by arrowhead on left) from a panel of human–rodent somatic cell hybrid DNA template. Std indicates ϕX174 DNA digested with *Hae* III; the sizes (bp) of relevant markers are indicated. Control templates are 1pt, representing 0.1 ng of human inositol polyphosphate 1-phosphatase cDNA; H_2O, representing no template added; 3T6 and CHO, representing DNA from parental mouse and hamster cell lines, respectively. Numbers above lanes indicate human chromosomes contained in each somatic cell hybrid.

The tissue distribution of human inositol polyphosphate 1-phosphatase mRNA was determined by Northern blot analysis (Fig. 2). Human mRNA from heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas was probed with the 1.74-kb human cDNA clone. A common 1.9-kb mRNA corresponding to full-length inositol polyphosphate 1-phosphatase was identified in all tissues tested; however, relative tissue amounts varied. Several higher molecular weight species of RNA were detected including a 7.1-kb species present in brain, skeletal muscle, heart, and liver; an 8.7-kb species observed in brain and liver; a 9.9-kb message present only in brain; an 11.7-kb RNA present in skeletal muscle.

The chromosome containing the human inositol polyphosphate 1-phosphatase gene was determined by a PCR assay of a panel of human–rodent somatic cell hybrid DNA. Sense and antisense primers specific to a 100-nucleotide region within the human cDNA coding sequence were used to amplify a product from DNA prepared from rodent cell lines harboring individual or multiple human chromosomes (Fig. 3). A specific product was amplified from a cell line containing human chromosome 2 (NIGMS strain GM10880); a faint product was found using NIGMS strain GM10880 containing chromosome 1 sequences; however, this line is documented to be contaminated with other unspecified human DNA sequences (NIGMS Human Genetic Mutant Cell Registration; unpublished observation).

Fluorescent *in situ* hybridization was used to corroborate the result obtained by PCR and to subregionally localize the human inositol polyphosphate 1-phosphatase gene. A human genomic DNA fragment was obtained by screening a Lambda FIX human lung fibroblast genomic library (7×10^5 recombinants) with the 100-bp region of human inositol polyphosphate 1-phosphatase cDNA obtained from PCR as described above. Three positive clones were identified and one of these, λG3-1 harboring a 12.2-kb genomic insert, was biotinylated and hybridized to prometaphase spreads of human chromosomes. The labeled DNA was detected with fluorescein isothiocyanate-conjugated avidin DCS and amplified using fluorescein isothiocyanate-conjugated goat anti-avidin D antibodies. Fifty independent metaphase spreads were analyzed and a representative example is shown (Fig. 4). Specific hybridization of the biotinylated human inositol polyphosphate 1-phosphatase genomic probe was observed on chromosome 2 (two metaphase chromosomes 2 are evident) and no consistent secondary hybridization was observed on any of the 50 spreads analyzed. 4,6-Diamidino-2-phenylindole dihydrochloride staining and Giemsa banding

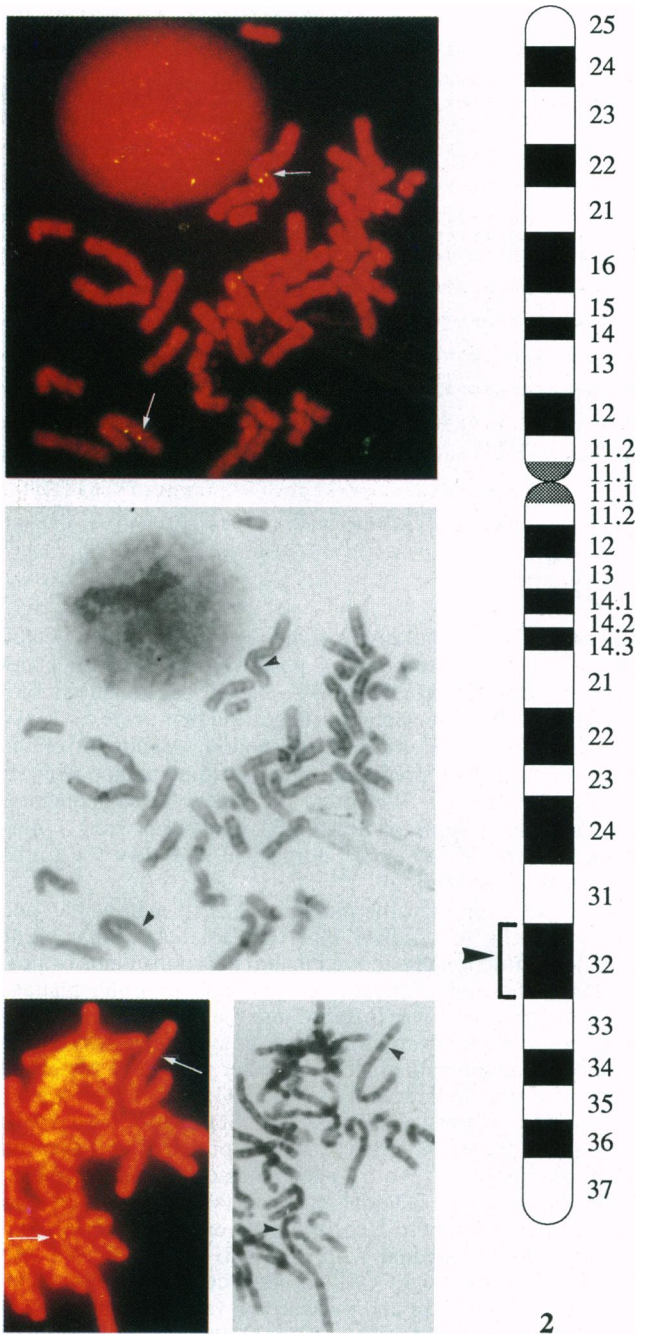

FIG. 4. Fluorescent *in situ* hybridization localization of the inositol polyphosphate 1-phosphatase gene to human chromosome 2 band q32. Fluorescence *in situ* hybridizations with a genomic clone, λG3-1, from the inositol polyphosphate 1-phosphatase gene locus. Two metaphase spreads are shown. Arrows indicated biotin-labeled probe hybridization (color) and the position on the same metaphase spreads banded using Giemsa dye. Also shown is an idiogram of chromosome 2 with the region 2 band q32, to which the probe hybridizes, indicated with an arrowhead.

(Fig. 4 *Bottom*) confirmed that the signals were located on chromosome 2 band q32. These data indicate that only one human gene exists and that the trace of 100-bp PCR product amplified from GM10880 DNA is a result of cross-contamination with human chromosome 2 sequences.

DISCUSSION

Human and bovine inositol polyphosphate 1-phosphatase exhibit 84% amino acid sequence identity. Especially highly conserved regions serve to identify functionally important regions. We previously described two sequence motifs conserved between inositol polyphosphate 1-phosphatase, inositol monophosphatase, and several bacterial and fungal genes of unknown but pleiotropic function (29, 39). The recently determined x-ray crystal structure of bovine inositol monophosphatase indicates that these two motifs are involved in the metal binding and the potential substrate binding sites. These regions are totally conserved in human and bovine inositol polyphosphate 1-phosphatase with 25 identical residues at the metal binding site (amino acids 144–168) and 28 identical residues at the putative substrate binding site (amino acids 303–330). An even larger stretch of 35 identical residues (amino acids 28–62) contains a potential nuclear localization signal (40, 41) (amino acids 36–44).

Human inositol polyphosphate 1-phosphatase is expressed in a variety of human tissues consistent with the distribution of the bovine enzyme (22). Several higher molecular weight mRNAs are also observed in brain, heart, liver, and skeletal muscle. These mRNAs may represent alternative isoforms of human inositol polyphosphate 1-phosphatase, incompletely processed mRNAs, or unique transcripts sharing significant homology to human inositol polyphosphate 1-phosphatase. Characterization of the genomic organization of human inositol polyphosphate 1-phosphatase in order to determine whether there are alternative exons within the gene remains to be done.

Human inositol polyphosphate 1-phosphatase is located at human chromosome 2 band q32. The chromosomal location of other genes involved in phosphatidylinositol metabolism is not known. Interestingly, inositol polyphosphate 1-phosphatase is inhibited by lithium at levels achieved intracellularly in patients undergoing lithium treatment (20, 21, 27). Epidemiological data and linkage analysis have suggested that a single gene may cause inherited depressive disorders; however, a gene locus has not been explicitly mapped (42–45). The human inositol polyphosphate 1-phosphatase gene is a candidate for linkage analysis in these patients.

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