Molecular Cloning, Expression, and Characterization of a Novel Human Serine/Threonine Protein Phosphatase, PP7, That Is Homologous to *Drosophila* Retinal Degeneration C Gene Product (rdgC)*

(Received for publication, July 25, 1997, and in revised form, October 8, 1997)

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A novel serine/threonine protein phosphatase (PPase) designated PP7 was identified from cDNA produced from human retina RNA. PP7 has a molecular mass of ~75 kDa, and the deduced amino acid sequence of PP7 contains a phosphatase catalytic core domain that possesses all of the invariant motifs of the PP1, PP2A, PP2B, PP4, PP5, and PP6 gene family. However, PP7 has unique N- and C-terminal regions and shares <35% identity with the other known PPases. The unique C-terminal region of PP7 contains multiple Ca\(^{2+}\) binding sites (i.e. EF-hand motifs). This region of PP7 is similar to the *Drosophila* retinal degeneration C gene product (rdgC), and PP7 and rdgC share 42.1% identity. Unlike the other known PPases, the expression of PP7 is not ubiquitous; PP7 was only detected in retina and retinal-derived Y-79 retinoblastoma cells. Expression of recombinant human PP7 in baculovirus-infected SF21 insect cells produces an active soluble enzyme that is capable of utilizing phosphohistone and \(p\)-nitrophenyl phosphate as substrates. The activity of recombinant PP7 is dependent on Mg\(^{2+}\) and is activated by calcium (IC\(_{50}\) = 250 \(\mu\)M). PP7 is not affected by calmodulin and is insensitive to inhibition by okadaic acid, microcystin-LR, calyculin A, and cantharidin.

In eukaryotes, the reversible phosphorylation of proteins catalyzed by protein kinases and protein phosphatases determines the biological activities of many proteins and is recognized as a major mechanism controlling cellular processes as diverse as cell cycle progression, metabolism, gene expression, and phototransduction. Traditionally, the protein phosphatases that catalyze the dephosphorylation of serine and threonine residues (PPases) have been classified into four subtypes (PP1, PP2A, PP2B, and PP2C) based on (a) their biochemical characteristics, (b) their sensitivities to specific inhibitors, and (c) a limited amount of substrate specificity that can be demonstrated in vitro (for review see Refs. 1 and 2). More recent studies indicate that the primary amino acid sequences of PP1, PP2A, and PP2B are related, whereas PP2C is structurally distinct and belongs to a completely different gene family (3, 4). In addition, multiple isoforms of all four PPases have been cloned. In mammals, three isoforms of PP1, which demonstrate >90% identity (5–7), two isoforms of PP2A with >97% identity (8–11), and three isoforms of PP2B with >80% identity (12–18) have been identified. Molecular studies have also identified three additional PPases designated as PP4 (PPX; Refs. 19 and 20), PP5 (21–23), and PP6 (PPV; Refs. 21 and 24). PP4 and PP6 are structurally related to PP2A, sharing 65 and 57% identity at the level of their primary amino acid sequence, respectively (19, 20, 24). PP5 contains a catalytic domain common to the PP1/PP2A/PP2B/PP4/PP6 family of enzymes and an extended N-terminal domain containing four 34-amino acid tetrapeptide repeat motifs (21–23).

The known PPases can also be classified based on their sensitivities to several natural toxins (25, 26). PP1, PP2A, PP4, and PP5 are all sensitive to okadaic acid and microcystin-LR (19, 22, 26–28). In contrast, PP2B is relatively resistant to the known inhibitors (okadaic acid, microcystin, nodularin, tautomycin, cantharidin, and calyculin A), and PP2C is insensitive to these compounds (4, 25–30). In addition, both PP2B and PP2C have an absolute requirement for divalent cations, which are not required by PP1, PP2A, PP4, or PP5. PP2C activity has an absolute requirement for Mg\(^{2+}\), and PP2B is activated by the association of a calcium-bound calmodulin complex (1, 2, 4). PP6 has not yet been characterized biochemically.

Several studies suggest that the expression of certain PPases, particularly isoforms of PP1, are altered in human tumor cells (31–33). Thus, the aim of this study was to characterize the heterogeneity of related PPases in Y-79 human retinoblastoma cells for comparison with those contained in normal human retina. In the course of these studies, we identified a novel human PPase that is directly activated by calcium and dependent on Mg\(^{2+}\) for activity.

**EXPERIMENTAL PROCEDURES**

PCR Amplification of Retinal cDNA—First strand human retinal cDNA was generously provided by Dr. S. Pittler and Dr. M. Ardell. Degenerate oligonucleotide primers matching to highly conserved regions of known PPases (RGNHE, DILWSDP, GDY/FVDR) were synthesized and employed in combination with an oligo\(dT\)\(_{15}\) primer to amplify human first-strand cDNA produced from human retina, Y-79 cells, and bovine brain by PCR. PCR reactions were allowed to proceed for 35 cycles (1 min at 95 °C, 45 s each at 40 and 45 °C; 1 min at 72 °C) in a solution containing 50 mM KCl, 2 mM MgCl\(_2\), 0.2 mM dNTP, 10 mM Tris-HCl, pH 8.3, and 100 ng of each primer. The PCR products pro-
duded were then analyzed by agarose gel electrophoresis, and ~125 bands were isolated and cloned into pT7/T3o-18 or pBluescript for sequence analysis.

Cloning of PP7—The PCR products were sequenced, and the sequences obtained were searched for homologous regions contained in known human and mouse genomic sequences, using the BLAST program. One sequence identifying a human cDNA clone isolated from fetal brain (GenBank™ accession number H18854) was homologous to the Drosophila retinal degeneration C gene product (rdgC). The human brain clone (ID51064) was obtained from Research Genetics, Inc. (Huntsville AL), and sequence analysis of this clone confirmed the similarity with rdgC (34). A 2.9 kb fragment from clone 51064 was radiolabeled (DECAprime II™, Ambion) and used as a probe to screen ~4 x 10⁶ clones from a human retina cDNA library constructed in Agt10. Four positive clones were isolated and completely sequenced.

Expression of Recombinant PP7 in Insect Cells—The full-length PP7 open reading frame was amplified with PCR employing a nested primer pair, (RH105, 5′-AATCTGCAATTGAGCTGGTCAGATCGG-3′, and RH112, 5′-ACCCGGATCCTGAGGTACGACCATTTCC-3′), which contained restriction sites (BamHI and PstI) added to the 5′ ends, respectively. The PCR product was then digested with BamHI/PstI and ligated into a baculovirus transfer vector, pFastBac1™ (Life Technologies, Inc.). The construct adds six consecutive His residues to the N-terminal region of PP7 to aid in the purification of the recombinant enzyme. After sequence- and promoter-terminating the insert, to confirm the fidelity of the construct, the recombinant plasmid was used to transform Escherichia coli (DH10Bac) containing a helper plasmid and bacmid. Recombinant bacmids were identified by the color phenotypes of the host E. coli colonies growing on Blue-gal plates (i.e. white color). Further confirmation was obtained by PCR analysis with PP7-specific primers. Approximately 5 μg of bacmid DNA was then purified and used to transfect CellFECTIN® (Life Technologies)-treated SF21 cells. Recombinant baculovirus (~9 x 10⁶) was harvested 72 h later, and the initial stock was reamplified and used to infect three 125-mm flasks of SF21 cells at a multiplicity of infection of 1. Cells were harvested 48 h after the infection, collected by centrifugation at 1000 rpm, sonicated in a binding buffer (5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.9, 2 mM phenylmethylsulfonyl fluoride), and subjected to ultracentrifugation at 400,000 g for 20 min. The supernatant was then passed over a Ni²⁺-charged chelating His-tag column (Novagen). The column was washed with 10 volumes of binding buffer and then with 6 volumes of washing buffer (60 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.9). The recombinant PP7 was then eluted with the addition of 3 column volumes of elution buffer (1 M imidazole, 1.5 M NaCl, 20 mM Tris-HCl, pH 7.9). Fractions containing PPase activity were pooled and concentrated and subjected to fast protein liquid chromatography gel filtration on a Superose 12 high resolution 10/30 column equilibrated with binding buffer. The active fractions were identified by absorbance at 280 nm and confirmed by assay for PPase activity. Active fractions were then pooled and subjected to a second round of affinity purification employing a Ni²⁺-charged chelatingHis-tag column as described above. The purity of the active fractions obtained were then analyzed by SDS-polyacrylamide gel electrophoresis.

RNA preparations and Northern blot analysis—Total RNA from Y-79 cell cultures or human retina was prepared using TRI reagent according to the methods of the manufacturer (Life Technologies, Inc.). Poly(A)⁺ RNA was obtained by affinity chromatography with an oligo(dT) spin column (PolyA)⁺ mRNA isolation kit; New England Biolabs). Poly(A)⁺ RNA was separated on formaldehyde-agarose (1.2%) gels and transferred to a nylon membrane (Duralon-U, Stratagene). The RNA was fixed to the membrane by ultraviolet irradiation (UV cross-linker; Stratagene). Northern blots containing 2 μg of Poly(A)⁺ RNA from eight different human tissues was purchased from CLONTECH. cDNA from human retina and Y-79 cells was produced from the appropriate mRNA (Y-79 mRNA was prepared as described above (Northern blot analysis), and human retina mRNA was purchased from CLONTECH) by incubating 1 μg of poly(A)⁺ RNA in a solution (20 μl total volume) containing 50 μM Tris-HCl, pH 8.3, 75 μM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, 0.5 mM dNTP mix, 100 ng of random primer, and 200 units of reverse transcriptase (Superscript II, Life Technologies, Inc.) at 42 °C for 50 min. After normalizing the concentration of Y-79 and human retina cDNA to that of the other tissues, 5 μl aliquots from each tissue type was employed as template in PCR reactions containing PP7 or GAPDH-specific primer pairs. The PCR reactions were carried out in a volume of 25 μl containing 200 μM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTP mix, 10 ng each of the primers, and 25 units of Taq DNA polymerase (Promega) on a PTC-100 programmable thermal cycler (MJ Research Inc.). The reaction mixture was denatured at 94 °C for 5 min, and the amplification reaction consisted of 25 (for GAPDH) or 38 (for PP7) cycles of denaturation (94 °C, 30 s), annealing (55 °C, 30 s), and extension (72 °C, 2 min), with a final extension at 72 °C for 10 min. The sequences for PP7 and GAPDH-specific primers pairs were 5′-CAGAGCATGAATGGGAA-CAGA-3′ and 5′-ACATGGCACGAAATCTTC-3′ and 5′-TGAAGTGCCGATCAGGTTTG-3′ and 5′-CATGGGGCCATAGG-TCCACC-3′. The PCR products were then resolved on a 2% agarose gel.

Phosphorylase assay—The activity of PP7 was assayed against [³²P]phosphorylase a, [³²P]phosphohistone, and pNPP. Phosphohistone and phosphorylase a were prepared as described by Honkanen et al. (34) and assayed as described by Critz and Honkanen (35). pNPP activity was measured at 30 °C and determined from the change in absorbance (A₂₈₀). Reactions were conducted in a final volume of 150 μl as described previously (30, 34). The reaction contained 50 mM Tris-HCl, pH 8.1 (or as indicated), 10 mM MgCl₂, 30 mM KCl, 20 μM pNPP, and the indicated concentrations of Ca²⁺. Reactions rates were determined using a Beckman DU 640 spectrophotometer over a 15-min period using ~0.5–2 µg of PP7. With both pNPP and phosphohistone, the concentrations of enzymes were adjusted to ensure that the dephosphorylation of substrate was <10% total available substrate, and the reaction was linear with respect to enzyme concentration and time.

Somatic Cell Hybrid Analysis—A human leukocyte genomic library constructed in λ EMBL-3 (CLONTECH) was screened using a λ-²³PIdATP-labeled cDNA probe complementary to ~200 bp of the C-terminal region of PP7 (bp 1998–2220). Phage DNA from λ clones that hybridized with the PP7 cDNA probe were then isolated using a Wizard λ DNA purification system (Promega) according to the method of the manufacturer. Sequence analysis of one positive clone revealed that it contains a complete exon encoding a portion of the C-terminal region of PP7. PCR with nested primers contained in this exon (5′-GGAAGAATTTCGTTGCATG-3′, sense, nucleotides 1999–2018; and 5′-TTAGGCAAGTTGGACTCATGAG-3′, antisense, nucleotides 2173–2186) amplifies a 199-bp intronless fragment of PP7 from human genomic DNA that is not amplified in rodent DNA. PCR analysis of a panel of human/rodent somatic cell hybrid DNA from the General Medical Sciences (NGMS) human/rodent somatic cell hybrid DNA mapping panel 2, Coriell Cell Repositories, Camden, NJ) was then employed to determine the chromosomal localization of PP7. Each 25-μl PCR reaction contained 50 mM Tris-HCl, pH 8.4, 50 mM NaCl, 1.5 mM MgCl₂, 0.1% Triton X-100, 200 μM dNTP, 100 ng of each primer, 25 unit of Taq DNA polymerase (Promega), and 100 ng of template DNA. DNA amplification was performed on a thermal cycler (Perkin-Elmer 2400) with 30 cycles of denaturation (94 °C, 30 s), annealing (55 °C, 30 s), and extension (72 °C, 1 min), with an initial denaturation (95 °C, 5 min) and a final extension (72 °C, 10 min). The products were analyzed by electrophoresis on 2.5% agarose gels. The same primers and PCR conditions were used to amplify PP7 from a panel of radiation hybrid DNAs that contain different portions of chromosome X (NGMS regional mapping panel for chromosome X, Coriell Cell Repositories, Camden, NJ).

RESULTS

Identification and Cloning of PP7—In an effort to characterize the PPase expressed in Y-79 retinoblastoma cells, several degenerate oligonucleotide primers corresponding to highly conserved regions of known PPases were constructed in the sense orientation. These primers were then employed in combination with oligo(dT)₃₄ antisense primers to amplify first-strand cDNA produced from human retina or Y-79 cell RNA employing essentially the same strategy developed by Wadzinski et al. (36). Electrophoretic analysis of the PCR products from ~200 reactions yielded numerous bands of different sizes, and ~125 were purified and ligated into pT7/T3o-18 or pBluescript. Systematic sequencing of these clones resulted in the identification of PP1a, PP1b, PP1y1, PP1y2, PP2aA, PP4,
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PP5, PP6 and several unidentified sequences (data not shown). To distinguish additional promising clones from the numerous PCR artifacts produced, we performed a BLAST computer search of the expressed sequence tag data base with the sequences of known PPases and those obtained via PCR. One sequence identified in the expressed sequence tag data base was a 875-bp fragment from human fetal brain (GenBank™ accession number H18854). This clone (ID51064) was obtained from Research Genetics, Inc. (Huntsville, AL), and the sequencing of clone 51064 confirmed the similarity with a DNA fragment produced by PCR. An 600-bp probe produced from clone 51064 was then constructed and used to screen a human retina cDNA library. After three rounds of purification, four positive clones were isolated and characterized by sequencing. All of the clones contained the same sequence, and the largest clone contained a 2,658-bp insert. Nucleotide sequence analysis of this clone revealed a 1,599-bp open reading frame flanked by a region containing 234 bp of the 5'-untranslated sequence upstream of a translation start site AGT/GCG that is compatible with the Kozak (37) consensus sequence. The clone, designated as PP7, also contains a 465-bp 3'-untranslated region ending in a poly(A) tail.

The PP7 open reading frame encodes a protein of 653 amino acids with a predicted molecular mass of 75 kDa. A computer-aided search for homology of the deduced amino acid sequence for PP7 with the GenBank™/EMBL data bank (GCG; Genetics Computer Group, Madison, WI) revealed greatest homology with the gene product encoded by the Drosophila retinal degeneration C (rdgC) gene (38). Direct comparison between PP7 and rdgC using the "gap routine" (GCG) indicates that they share 42.1% identity and 54.3% similarity when conservative substitutions are considered (Fig. 1). In addition, two putative functional domains were identified through a motif search using "motif routine" (GCG). One is a serine/threonine phosphatase catalytic core domain, which is located between amino acids 166 and 436. This core is shared by PP1-PP6 (39, 40), and the overall identity of PP7 is 28–35% with these PPases. The similarity increases to 35–44% when conservative substitutions are considered. In addition, PP7 contains all 53 of the amino acids that are absolutely conserved in PP1-PP6 (Fig. 2) and all of the 16 amino acids predicted to form the active site of these enzymes based on the crystal structure of PP1 (40).

The second domain identified in PP7 is comprised of putative Ca2+ binding EF-hand motifs located in the C-terminal region. Two EF-hand motifs (amino acids 579–591 and 619–631) are excellent matches to the consensus sequence (41), and three other EF-hand-like motifs with weaker identity were recognized in the PP7 sequence (Figs. 1 and 2). These EF-hand and EF-hand-like motifs are conserved between PP7 and rdgC (Fig. 2). PP7 also contains an inserted domain made up of 43 amino acids (305–347) in the PPassic core region that is not found in PP1-PP6. The rdgC gene sequence predicts that rdgC contains a 15-amino acid insert in a corresponding region, and 6 of the 15 amino acids in the rdgC insert are conserved in PP7. The N-terminal region of PP7 is structurally divergent from that of PP1-PP6 (Fig. 2).

**Figure 1. Sequence similarity between the Drosophila retinal degeneration C gene product (DrdgC) and PP7.** The sequences of PP7 and the putative phosphatase deduced from the amino acid sequence of the retinal degeneration C gene (Ref. 38; accession number M98628) are aligned. Identical residues are shaded, and conserved replacements are indicated by an * below the sequence. The EF-hand and EF-hand-like motifs predicted to form calcium binding domains are underlined. Based on their primary amino acid sequences, PP7 and rdgC share 42.1% identity and 54.3% similarity when conservative substitutions are considered.

*Properties of PP7 Expressed in SF21 Insect Cells—To determine whether PP7 possesses endogenous phosphatase activity, full-length PP7 was overexpressed in SF21 insect cells. The addition of a His tag to the N terminus of PP7 allowed for a single-step purification to ~80% homogeneity using a Ni2+ chelating column. Further purification was achieved by gel filtration chromatography employing fast protein liquid chromatography with a Superose-12 column, and purification to apparent homogeneity was then achieved by a second round of affinity chromatography using a Ni2+ -chelating column.**

**Figure 2. Tissue Distribution and Expression of PP7 mRNA—The expression of PP7 poly(A)+ RNA in human heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, retina, and Y-79 retinoblastoma cells was analyzed by PCR amplification of normalized human cDNA and Northern analysis.** PP7 mRNA was only detected in retina and Y-79 cells by PCR analysis, suggesting that PP7 is not ubiquitously expressed like PP1, PP2A, PP4, or PP5. Northern analysis employing 2 μg of poly(A)+ RNA did not identify PP7 mRNA in any of the tissues tested, identifying a single ~2.8-kilobase transcript only in mRNA produced from Y-79 cells (Fig. 3B). When 10 μg of poly(A)+ RNA was employed, a second minor transcript (~4.4 kilobases) was also detected (Fig. 3C).

**Properties of PP7 Expressed in SP21 Insect Cells—To determine whether PP7 possesses endogenous phosphatase activity, full-length PP7 was overexpressed in SF21 insect cells. The addition of a His tag to the N terminus of PP7 allowed for a single-step purification to ~80% homogeneity using a Ni2+ -chelating column.** Further purification was achieved by gel filtration chromatography employing fast protein liquid chromatography with a Superose-12 column, and purification to apparent homogeneity was then achieved by a second round of affinity chromatography using a Ni2+ -chelating column. Purified PP7 displays a molecular mass of ~75 kDa after SDS-polyacrylamide gel electrophoresis (Fig. 4), which is in agreement with the calculated molecular mass from the amino acid sequence.

Recombinant PP7 is soluble and has activity against histone phosphorylated by cAMP-dependent protein kinase and pNPP, which is dependent on Mg2+ and activated by Ca2+ (Fig. 5). No activity was detected against phosphohistone and pNPP, and activity against both phosphohistone and pNPP in the absence of Ca2+ (Fig. 5). However, no activity is observed in the absence of Mg2+. The addition of calcium results in a dose-dependent increase in the activity of recombinant PP7, with ~250 μM Ca2+ producing half-maximal stimulation (Fig. 5). Maximal stimulation occurs at calcium concentrations between 400 and 500 μM, and no additional increase in activity is observed upon
the addition of >5 mM Ca\(^{2+}\). The addition of calmodulin to the assay had no detectable effect on the activation of PP7 by Ca\(^{2+}\) with either substrate. The activity of PP7 is sensitive to pH but not affected by several natural toxins with inhibitory activity against PP1 and PP2A. Little activity is observed under acid (pH < 6) or alkaline (pH >10.5) conditions, and maximal activity is observed at a pH of ~8.0. The activity of PP7 is insensitive to 1 μM okadaic acid, 100 nM microcystin-LR, 100 nM calyculin A, or 20 μM cantharidin, which completely inhibits the activity of PP1, PP2A, PP4, and PP5 (19, 22, 25, 26, 28, 30).

Chromosomal Localization of PP7—To determine the chromosomal localization of PP7, DNA from a human/rodent somatic cell hybrid mapping panel (panel 2 from Coriell NIGMS human genetic mutant cell repository, Camden, NJ) was analyzed for the presence of the human PP7 gene by PCR. A PP7-specific human fragment of ~200 bp was present only in hybrid GM/NA06317. This indicates that PP7 maps to chromosome X. PCR analysis of a regional mapping panel of human chromosome X (Coriell NIGMS Human genetic mutant cell repository, Camden, NJ) confirmed the presence of PP7 on chromosome X and assigned the gene for PP7 to Xp21.1-pter.

DISCUSSION

The human PP7 cDNA sequence presented here encodes a novel human serine/threonine PPase. PP7 possesses all of the invariant motifs found among members of the PP1/PP2A/PP2B/PP4/PP5/PP6 family of PPases that are believed necessary for catalytic activity, suggesting that PP7 belongs to this superfamily of PPases (39, 40). The PP7 open reading frame encodes a protein of 653 amino acids with a predicted molecular mass of ~75 kDa, which is consistent with the migration rate on SDS-polyacrylamide gel electrophoresis observed with recombinant PP7 produced in SF21 cells (Fig. 4). In comparison, the molecular masses of PP1, PP2A, PP2B, PP4, PP5, and PP6 are 37, 36, 61, 35, 58, and 36 kDa, respectively. Unlike PP1, PP2A, PP4, and PP5, PP7 is not sensitive to okadaic acid, microcystin-LR, calyculin A, or cantharidin, and PP7 contains an extended C-terminal region possessing five putative high affinity calcium binding domains referred to as EF-hand and EF-hand-like motifs. The presence of EF-hand motifs suggests that calcium directly regulates the activity of PP7, and indeed, studies with recombinant PP7 demonstrate that PP7 is activated by Ca\(^{2+}\). Like PP7, PP2B is also activated by calcium and is insensitive to okadaic acid and microcystin-LR. However, unlike the Ca\(^{2+}\) activation of PP2B, which is mediated by the interaction of a 19-kDa Ca\(^{2+}\)-bound calmodulin complex (referred to as calcineurin B or the B regulatory protein of PP2B) with a specific calmodulin binding domain located in the C-terminal region of PP2B (16, 42), the activation of PP7 by calcium appears to be direct because 1) PP7 lacks the calmodulin binding domain, 2) calmodulin has no apparent effect on its C-terminal region and two small divergent regions (indicated by open boxes) in the catalytic domain near the okadaic acid/microcystin binding domain, PP5 possesses four tetratricopeptide (TPR) domains in the N-terminal region. PP7 differs from all of the other PPase families in that it contains EF-hand motifs in the C-terminal region (indicated by filled squares) and a 43-amino acid insert in the catalytic core domain (indicated by an open box). B, sequence similarity between the catalytic core domain of PP7 and other human PPases. Residues that are identical in all of the PPases compared are indicated in boldface type. PP2A and PP2B have 80% sequence identity.
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PP7 activity, and 3) the activity of PP7 is increased with the addition of Ca\(^{2+}\) in the absence of calmodulin (Fig. 5). Therefore, PP7 is both structurally and biochemically distinct from all known mammalian PPases.

Comparison of the deduced amino acid sequence for PP7 with the GenBank\textsuperscript{TM}/EMBL data bank reveals the greatest homology with the gene product encoded by the Drosophila retinal degeneration C gene (rdgC). A direct comparison of PP7 and rdgC indicates that they share 42.1% identity and 54.3% similarity (38), which is greater than the homology shared between PP7 and any other mammalian PPases. This is unlike the other known PPases, such as PP1 and PP2A, which are more highly conserved between man and fly, sharing 89 and 94% identity, respectively. Nonetheless, like PP7, rdgC contains both the PPase catalytic core domain and the five putative Ca\(^{2+}\) binding EF-hand motifs (Fig. 1). Both PP7 and rdgC also contain a region within the catalytic core domain that is not observed in any of the other PPases (Fig. 2). The function of this inserted region in PP7 or rdgC is not known. However, considering the homology of PP7 and the rdgC gene product, it is likely that PP7 is the human homologue of Drosophila rdgC.

The physiological functions of PP7 remain unknown. PCR and Northern blot analyses only detected PP7 mRNA in retina and Y-79 cells, and mRNA encoding PP7 is not nearly as abundant as mRNA encoding PP1 or PP2A. However, an expressed sequence tag clone encoding part of PP7 was produced from human fetal brain, indicating that PP7 expression may also occur in the brain during development. The higher level of PP7 expression in Y-79 cells, as compared with retina, may also implicate PP7 in the neoplastic transformation of human retina. Alternatively, the PP7 mRNA may be unstable and the increased detection could simply reflect the ability to obtain higher quality mRNA from cultured cells as compared with human tissue, which cannot be acquired as rapidly. Nonetheless, unlike PP1, PP2A, PP4, and PP5, the expression of PP7 is not ubiquitous, suggesting that the functions of PP7 are more limited and possibly confined to the retina and brain.

In Drosophila, mutations in the rdgC gene are associated...
with light-induced retinal degeneration (38, 43). In the wild type fly, light-induced elevation of cytosolic Ca^{2+} during illumination is required to ensure the dephosphorylation of phosphorylated rhodopsin and the smooth operation of the phototopic cycle (44). Studies with rdgC mutants indicate that the PPase encoded by the rdgC gene is activated by Ca^{2+} and utilizes phosphorylated rhodopsin as a major substrate (38, 44). Such studies also indicate that photoreceptor degeneration occurs when excessive light-dependent phosphorylation of the phototopigment is unbalanced due to the lack of normal dephosphorylation by the rdgC PPase (44).

In humans, retinitis pigmentosa (RP) is the name given to a heterogeneous group of genetic disorders that result in the degeneration of the photoreceptor cells, and there are striking similarities between Drosophila retinal degeneration mutants (rdgC mutants) and vertebrate RP (43, 45). For example, in Drosophila the gene products encoded by rdgA or rdgB (diacylglycerol kinase and phosphatidylinositol transfer protein, respectively) are essential components of the phototransduction process. Mutations in either rdgA or rdgB result in retinal degeneration, indicating that several forms of RP and associated disorders map to Xp21.1, Xp21.3-p21.2, Xp22.13-p22.11, and Xp21-p22, respectively (51, 52). Thus, PP7 is a candidate gene for all of these disorders.

To date, linkage studies have identified more than 15 genes associated with human RP, and many of the known mutations in RP patients affect genes encoding proteins that are important for phototransduction (49, 50). In the mammalian photoreceptor both phosphorylation and the regulation of intracellular calcium levels are key factors in the phototransduction cascade, influencing the sensitivity and kinetics of several steps of both the excitatory and recovery processes (43, 45). Therefore, the physiological functions of PP7 in either the recovery or adaptation processes are not well defined. In vitro studies indicate that phosphoprotein is a substrate for PP2A (56–58), and PP1, which does not utilize phosphoprotein as a substrate, may dephosphorylate the cGMP-gated cation channel (55). Nonetheless, both PP1 and PP2A demonstrate little substrate specificity in vitro, whereas in vivo, the substrate specificity of these PPases is mediated via the association with regulatory or substrate targeting proteins (4). Therefore, the physiological significance of studies conducted with purified catalytic subunits of PPases in vitro must be interpreted with caution (1, 2, 4, 25). Clearly, a major challenge for future studies will be to determine the physiological substrates for PP1, PP2A, and PP7 in vivo and to determine the role of PP7 in retinitis pigmentosa or related disorders.

Acknowledgments—We thank Drs. M. Ardell and S. Pittler and for their kind and constructive criticism, Jamie Koons for expert technical assistance, and Dr. Pittler for an aliquot of Agt11 human retina cDNA library.

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