We have isolated the cDNAs encoding human and mouse homologues of a yeast protein, termed peroxisomal membrane protein 20 (PMP20). Comparison of the amino acid sequences of human (HsPMP20) and mouse (MmPMP20) PMP20 proteins revealed a high degree of identity (93%), whereas resemblance to the yeast Candida boidinii PMP20A and PMP20B (ChbPMP20A and ChbPMP20B) was less (30% identity). Both HsPMP20 and MmPMP20 lack transmembrane regions, as do ChbPMP20A and ChbPMP20B. HsPMP20 mRNA expression was low in human fetal tissues, especially in the brain. In adult tissues, HsPMP20 mRNA was expressed in the majority of tissues tested. HsPMP20 and MmPMP20 contained the C-terminal tripeptide sequence Ser-Gln-Leu (SQL), which is similar to the peroxisomal targeting signal 1 utilized for protein import into peroxisomes. HsPMP20 bound directly to the human peroxisomal targeting signal 1 receptor, HsPEX5. Mutagenesis analysis showed that the C-terminal tripeptide sequence, SQL, of HsPMP20 is necessary for its binding to HsPEX5. Subcellular fractionation of HeLa cells, expressing epitope-tagged PMP20, revealed that HsPMP20 is localized in the cytoplasm and in a particulate fraction containing peroxisomes. Double-staining immunofluorescence studies showed colocalization of HsPMP20 and thiolase, a bona fide peroxisomal protein. The amino acid sequence alignment of HsPMP20, MmPMP20, ChbPMP20A, and ChbPMP20B displayed high similarity to thiol-specific antioxidant proteins. HsPMP20 exerted an inhibitory effect on the inactivation of glutamine synthetase in the thiol metal-catalyzed oxidation system but not in the nonthiol metal-catalyzed oxidation system, suggesting that HsPMP20 possesses thiol-specific antioxidant activity. In addition, HsPMP20 removed hydrogen peroxide by its thiol-peroxidase activity. These results indicate that HsPMP20 is imported into the peroxisomal matrix via PEX5p and may work to protect peroxisomal proteins against oxidative stress. Because some portion of PMP20 might also be present in the cytosol, HsPMP20 may also have a protective effect in the cytoplasm.

Peroxisomes, also called microbodies, are single-membrane-bound organelles present in all mammalian cells with the exception of erythrocytes and are also found in plants, yeast, and most other eukaryotic cells. The peroxisome contains nearly 50 enzymes, many participating in various metabolic pathways (1, 2). Human peroxisomal enzymes are involved in numerous metabolic processes including β-oxidation of long and very long chain fatty acids, several steps in the synthesis of ether lipid, bile acids, and cholesterol, oxidation of d-amino acids, and α-oxidation (2, 3). Peroxisomes also contain catalase, which plays a central role in eliminating the hydrogen peroxide (H₂O₂) produced by peroxisomal oxidases.

“Newly synthesized” peroxisomal matrix proteins contain a peroxisomal targeting signal (PTS),¹ either PTS1 (4) or PTS2 (5), and are imported post-translationally (6) from the cytoplasm into the peroxisomes by the PTS1 and PTS2 receptors, respectively (7). The PTS1 sequence is a C-terminal tripeptide, Ser-Lys-Leu (SKL) or a variant (4), whereas the PTS2 sequence is an N-terminal peptide, R/K/(L/V/I)X₅(H/Q)/(L/A) (5). Most peroxisomal matrix proteins utilize PTS1, whereas a few utilize PTS2. However, either sequence is sufficient for peroxisomal targeting and is used by evolutionarily diverse organisms (8).

Yausto peroxisome biogenic mutants (pex mutants) have been used to identify over 20 genes (PEX) and their protein products (peroxins) that are required for peroxisomal protein import and biogenesis (8, 9). These genes include PEX5 and PEX7, encoding the receptors for PTS1 and PTS2 sequences, respectively (10). Although human PEX5 isoforms are mainly present in the cytoplasm (11, 12), they shuttle between the cytosol and the peroxisomal membrane, bringing the PTS1-containing proteins into the peroxisomes (7, 13).

For some peroxisomal proteins, no apparent functions have been defined. Among these are the yeast PMP20 proteins. A database search revealed that Candida boidinii PMP20 and the Saccharomyces cerevisiae counterpart contained the PTS1 sequences, Ala-Lys-Leu (AKL) and Ala-His-Leu (AHL), respectively. In addition, secondary structure analysis of all yeast PMP20 proteins reported suggests that there are no obvious membrane-spanning regions as previously reported for ChbPMP20A and ChbPMP20B (14). Initially, the C. boidinii PMP20 was defined as a membrane protein (15, 16). However, PMP20 is released from the membrane and was shown to be

¹ The abbreviations used are: PTS, peroxisomal targeting signal; PMP, peroxisomal membrane protein; PCR, polymerase chain reaction; EST, expressed sequence tag; GST, glutathione S-transferase; TSA, thiol-specific antioxidant; DTT, dithiothreitol; MCO, metal-catalyzed oxidation; Hs, Homo sapiens; Mm, Mus musculus; Ch, C. boidinii; Sc, S. cerevisiae; bp, base pair(s); HA, hemagglutinin; GFP, green fluorescent protein; TK, thymidine kinase; EGF, epidermal growth factor; NRP/B, nuclear matrix protein B; PAGE, polyacrylamide gel electrophoresis.
present in the matrix by immunocytochemistry (17–23). In the present study, we have cloned two mammalian PMP20 cDNAs, determined the subcellular location of the PMP20 protein in mammals, and discovered a potential function for this class of proteins.

**EXPERIMENTAL PROCEDURES**

**Cloning and Sequencing of Human PMP20 cDNA—**When a nonredundant data base of human expressed sequence tag (EST) entries in GenBank was screened for human cDNAs similar to the CbPMP20A sequence, no significant matches were found. Screening EST databases (e.g., EST179427, EST185495, and EST186764) displayed some similarity to the coding region of the CbPMP20A sequence. Using the EST179427 sequence, we designed primers and amplified the putative sequence by the polymerase chain reaction (PCR) using the template DNA from a human hippocampus cDNA library in the ZAP II vector (Stratagene, San Diego, CA). The PCR fragment was sequenced to confirm identity (99%) with the EST179427 sequence. The PCR fragment was radiolabeled using an α-32PdCTP (NEN Life Science Products). Using this probe, the human hippocampus cDNA library was screened by hybridization. Positive clones were isolated, plaque-purified (37), and DNA was extracted following manufactures’ instructions. In addition, each blot was probed for human PMP20 protein (PMP20-WT) fused with GST at the N terminus, as described previously (30). The reaction was initiated by adding 10 mM isopropyl β-D-thiogalactopyranoside (U. S. Biochemical Corp.) induction, and purified using glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech) according to the manufacturer’s protocol. HsPMP20 was cleaved from the GST using thrombin (Amersham Pharmacia Biotech) according to the manufacturer’s instructions.

**Northern Blot Analysis—**Blots containing poly(A)+ RNA from various human fetal and adult tissues were purchased from CLONTECH (Palo Alto, CA). A gene-specific probe was generated by restriction digestion of the human PMP20 (HsPMP20) cDNA by PstI (nucleotides 218–683). This 460-bp fragment was radiolabeled to a specific activity of 106–107 cpm/μg and was used as a probe for all Northern blots. The blots were hybridized with the probe according to the manufacturer’s instructions. In addition, each blot was probed for β-actin or glyceraldehyde-3-phosphate dehydrogenase.

**Vectors, Antibodies, Cell Culture, and Transfection—**The DNA corresponding to the HA tag sequences (50 bp) and the coding region of human PMP20 cDNA (486 bp) were subcloned into the pcDNA3 vector (Invitrogen, Carlsbad, CA) to yield pcDNA3-HA-HsPMP20. We subcloned the PMP20 cDNA into the pTK-Hyg vector (CLONTECH, Palo Alto, CA) and fused it to the gene encoding the green fluorescent protein (GFP) under the control of the herpes simplex virus thymidine kinase (TK) promoter, to generate the plasmid pTK-GFP-HsPMP20-Hyg. The cloned PMP20 cDNA into the pTK-Hyg vector (CLONTECH, Palo Alto, CA) and fused it to the gene encoding the green fluorescent protein (GFP) under the control of the herpes simplex virus thymidine kinase (TK) promoter, to generate the plasmid pTK-GFP-HsPMP20-Hyg. The vector sequence was confirmed by DNA sequencing. GST fusion proteins were produced via isopropyl β-D-thiogalactopyranoside (U. S. Biochemical Corp.) induction, and purified using glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech) according to the manufacturer’s protocol. HsPMP20 was cleaved from the GST using thrombin (Amersham Pharmacia Biotech) according to the manufacturer’s instructions.

**Cloning and Characterization of Human PMP20—**Northern blots of human fetal and adult tissues were purchased from CLONTECH (Palo Alto, CA). A gene-specific probe was generated by restriction digestion of the human PMP20 (HsPMP20) cDNA by PstI (nucleotides 218–683). This 460-bp fragment was radiolabeled to a specific activity of 106–107 cpm/μg and was used as a probe for all Northern blots. The blots were hybridized with the probe according to the manufacturer’s instructions. In addition, each blot was probed for β-actin or glyceraldehyde-3-phosphate dehydrogenase.

**Preparation of GST Fusion Proteins and Far Western Analysis—**For precipitation of GST fusion proteins, COS-7 cells transfected with pcDNA3-HsPEX5 were lysed, and 10 μg of various GST fusion proteins were used to bound glutathione-Sepharose 4B beads. Bound proteins were separated by SDS-PAGE and immunoblotted with anti-PEX5 or anti-GST antibodies. For Far Western analysis, purified HsPMP20, GST fusion proteins containing HsPMP20-WT and HsPMP20-QSLL, or GST alone were separated by 10% SDS-PAGE, transferred onto a nitrocellulose membrane, and subjected to Far Western blotting, using biotinylated HsPMP20 (25).

**Cell Fractionation—**The subcellular location of HsPMP20 was determined by cell fractionation (27). HeLa cells transiently transfected with pcDNA3-HA-HsPMP20 or pcDNA3-HA-HsPMP20 were washed with phosphate-buffered saline, resuspended in a hypotonic solution, passed through a 30-gauge needle, and centrifuged at 600 × g for 10 min to collect crude nuclei. These nuclei were further purified and used as the nuclear fraction. The supernatant was centrifuged at 10,000 × g for 10 min to collect the heavy membrane fraction (mitochondria, lysosomes, and peroxisomes). The supernatant was further centrifuged at 100,000 × g for 90 min, and the pellet and supernatant were used as the light membrane fraction (plasma membrane and microsomes) and cytoplasmic fraction, respectively. HeLa cells were standardized to represent an equal number of cells in each fraction and analyzed by SDS-PAGE and immunoblotting with anti-PEX5, anti-HA, anti-catalase, anti-peroxisomal thiolase, anti-EFG receptor, and anti-NRP/PB antibodies.

**Immunofluorescence labeling was performed as described previously (26). Briefly, HeLa cells transfected with pcDNA3-HA-HsPMP20 or pcDNA3-HA-HsPMP20 were grown in chamber slides (Lab-Tek, Naperville, IL). Adherent cells were fixed with neutral buffered 4% (w/v) paraformaldehyde and then permeabilized with 0.5% Triton X-100. Double-label immunofluorescent staining was performed using mouse anti-HA antibody followed by goat anti-mouse IgG-fluorescein isothiocyanate (Vector Labs, Burlingame, CA) to decorate PMP20 in the HeLa cells. Peroxisomes were decorated with rabbit anti-thiolase antibody and donkey anti-rabbit Red (Jackson ImmunoResearch, West Grove, PA). Immunostained preparations were examined using a Leica TCSNT confocal laser scanning microscope (Leica Inc., Exton, PA) fitted with air-cooled Argon and Krypton lasers. Fields of view were selected and brought into view under bright-field imaging conditions. Confocal micrographs of emission spectra (530 ± 15 nm and ≥590 nm) were recorded under dual-channel fluorescence imaging mode using excitation wavelengths of 488 and 568 nm. Images were collected from a 100× oil objective lens with 0.02 micron pixel size. Micrographs were examined using ImageSpace software (Molecular Dynamics, Sunnyvale, CA).

**Assay of Antioxidant Activity of Human PMP20—**The antioxidant activity of HsPMP20 was determined by monitoring the ability of the protein to inhibit the inactivation of glutathione synthetase (Sigma) by a thiold-catalyzed MCO system as described previously (28, 29). The assay was performed in a 50-μl reaction containing 50 mM imidazole-HCl (pH 7.0), 5 μg of glutathione synthetase, 3 μM FeCl3, 10 mM dithiothreitol (DTT) and either 1 mM EDTA or 0–0.18 mg/ml HsPMP20 protein. For the nonthiol MCO system, DTT was replaced with 10 mM ascorbic acid. Following incubation at 30°C for the indicated periods, the remaining activity of glutathione synthetase was measured by adding 5 μl of the reaction mixture to 2 ml of the peroxidase assay mixture as described (28). The peroxidase activity of HsPMP20 was assayed as described previously (30). The reaction was initiated by adding 10 mM H2O2 to a 100-μl reaction containing 0.25 mM DTT, 0.15 mg/ml HsPMP20, 100 mM NaCl, and 50 mM HEPES (pH 7.0) at 37°C. The concentration of the H2O2 remaining at the indicated time points was measured by the thiocyanate method as described previously (30).

**Preparation of GST Fusion Proteins—**To construct wild-type human PMP20 protein (PMP20-WT) fused with GST at the N terminus, the coding region of HsPMP20 cDNA was amplified by PCR using Fhu DNA polymerase (Stratagene) and the following forward and reverse primers: 5′−ATG GCC CCA ACA TAC AAG GTG GGA−3′, 5′−GCC TCA GAG CTG TGA GAT GAT−3′ with the attached restriction enzyme sites, BamHI and XhoI, respectively. The DNA (∼500 bp) fragment obtained from the PCR was gel-purified, digested, and ligated into the pGEX-4T-2 vector (Amersham Pharmacia Biotech). Constructs of mutant PMP20 proteins fused with GST were constructed using PMP20 CDS and the degenerate oligonucleotide primer set—(500 μg/sample) were immunoprecipitated with anti-HA antibody, anti-PEX5 antibodies, anti-GST antibody, or normal mouse IgG. Immunoprecipitates were separated by SDS-PAGE and transferred onto PVDF-Plus membranes (Micron Separations Inc., Westboro, MA). Bound proteins were immunoblotted with either anti-HA antibody or with anti-PES5, as described (25). The blots were developed using enhanced chemiluminescence reagent (Amersham Pharmacia Biotech). Electrophoresis reagents were obtained from Bio-Rad.
RESULTS

Human PMP20 Is Homologous to the Yeast PMP20—Because mammalian cDNA for PMP20 had not been described, we used the EST179427 sequence to probe a human hippocampus cDNA library and obtained four clones ranging in length from 0.45 to 1 kilobase pair. The longest cDNA (approximately 850 bp without the poly(A) stretch) encodes a protein consisting of 162 amino acids with an estimated molecular mass of 20 kDa (Fig. 1A). The sequence is likely to represent the full-length cDNA, because no cDNAs with a longer 5′-flanking region could be isolated. The codon at base pairs 226–228, along with the flanking nucleotides, correspond to the consensus sequence for an optimal translation initiation site (31, 32). The mouse PMP20 (MmPMP20) cDNA was cloned from a mouse brain library using the HsPMP20 cDNA as a probe. HsPMP20 and MmPMP20 share 88% nucleotide identity and 93% amino acid identity, and both have coding regions of 162 amino acids. The C-terminal tripeptide sequence of both HsPMP20 and MmPMP20 was Ser-Gln-Leu (SQL).

A search using the HsPMP20 polypeptide against GenBank™ via the BLAST 2.0 and FASTA programs detected a similarity with yeast PMP20 proteins, CbPMP20A, CbPMP20B, and ScPMP20. Alignment of the amino acid sequences of HsPMP20 with ScPMP20 and CbPMP20A revealed 67 and 65% similarity, respectively (Fig. 1B). The overall amino acid sequence identities between HsPMP20 and ScPMP20 or CbPMP20A were 27 and 35%, respectively (Fig. 1B). These proteins are of similar length and are more similar to each other than to any other known proteins. Based on this sequence similarity, it is most likely that HsPMP20, MmPMP20, ScPMP20, and CbPMP20A are homologous. Analysis of HsPMP20 and MmPMP20 using the Prosite data base revealed no glycosyla-
FIG. 2. Expression of the HsPMP20 gene in various human tissues. A, expression of human PMP20 in human fetal tissues by Northern blot analysis. B and C, expression of human PMP20 in human adult tissues by Northern blot analysis. The RNA blots were hybridized with an α-32P-labeled human PMP20 gene-specific probe, followed by hybridization with β-actin or glyceraldehyde-3-phosphate dehydrogenase probes as controls for uniform RNA loading. Shk. Musc., skeletal muscle; Sm. Intestine, small intestine; PBL, peripheral blood leukocytes.

Subcellular Localization of HsPMP20—Cell fractionation of HeLa cells, expressing an HA-tagged PMP20, revealed that HsPMP20 protein localizes in a fraction enriched in mitochondria, lysosomes, and peroxisomes, and in the cytoplasmic fraction (Fig. 3). As expected, HsPEX5 was located mainly in the cytoplasmic fractions and partly in the heavy membrane fraction (Fig. 3). Similar results were obtained with HeLa cells expressing GFP-HsPMP20 (data not shown). Antibodies to peroxisomal catalase and 3-ketoacyl-CoA thiolase were used as controls. Both markers were localized in the heavy membrane fraction (Fig. 3) and were also faintly detected in the cytosolic fractions, consistent with their known ability to leak out from peroxisomes during tissue homogenization (11). As expected, the EGF receptor and NRP/B nuclear matrix protein were mainly localized in the light membrane and nuclear fractions, respectively (Fig. 3).

Double-staining immunofluorescence studies using HeLa cells that were transfected with either pcDNA3-HA-HsPMP20 or pTK-GFP-HsPMP20-Hyg revealed HA-HsPMP20 in punctate structures (Fig. 4, B and C), and there was very strong colocalization of HsPMP20 with thiolase (Fig. 4, G and I), as well as with catalase (data not shown). Thus, these results support the conclusion of the biochemical analysis (Fig. 3) and show that the epitope-tagged HsPMP20 colocalizes with genuine peroxisomal matrix proteins.

Association of HsPMP20 with HsPEX5—The PTS1 sequence is known to be SKL or a variant (4). Because the C-terminal tripeptide sequence of HsPMP20 was similar to SKL, the ability of HsPMP20 to bind to the human PTS1 receptor, HsPEX5, was examined. HsPMP20 fused at its N terminus with an HA epitope (HA-HsPMP20) was expressed together with HsPEX5 in COS-7 cells upon transient transfection. Cells co-transfected with the control vector (pcDNA3-HA) or pcDNA3-HA-HsPMP20 together with pcDNA3-HsPEX5 were lysed and immunoprecipitated with mouse anti-HA monoclonal antibodies, rabbit anti-Pex5 antibodies, or control antibodies. Bound proteins were analyzed by Western blotting. When both HsPMP20 and HsPEX5 were expressed, anti-HA antibodies co-immunoprecipitated HsPMP20 (Fig. 5A). In addition, anti-Pex5 polyclonal antibodies co-immunoprecipitated HA-HsPMP20 (Fig. 5B). These results indicate that HsPMP20 and HsPEX5 proteins interact in cells.

Direct Binding of HsPEX5 to the C-terminal Tripeptide Sequence of HsPMP20—To examine whether HsPMP20 and HsPEX5 can directly bind to one another, Far Western blotting was employed. Purified HsPMP20, bovine serum albumin, and GST fusion proteins were blotted onto a nitrocellulose membrane and subjected to Far Western analysis (Fig. 6A, upper panel). The biotinylated HsPMP20 protein could bind to both HsPMP20 (Fig. 6A, lower panel, lane 1) and HsPEX5 fused at the N terminus with GST (Fig. 6A, lower panel, lane 3). The biotinylated HsPEX5 protein could not be bound to the GST fusion protein lacking the C-terminal tripeptide (HsPMP20ΔSQL) (Fig. 6A, lower panel, lane 4) or to GST alone (Fig. 6A, lower panel, lane 5). To determine the importance of the SQL motif, COS-7 cells transfected with HsPEX5 cDNA were lysed and precipitated with various GST fusion proteins. Co-precipitates were separated by...
SDS-PAGE and immunoblotted with anti-HsPEX5 antibodies. GST fusion proteins containing the wild-type and the mutant HsPMP20, whose C-terminal tripeptide SQL was replaced with SKL (PMP20Q161K), also precipitated HsPEX5 (Fig. 6B, upper panel). PMP20SQL or GST alone failed to precipitate HsPEX5. The amount of GST fusion proteins used for the assay was similar (Fig. 6B, lower panel). These results indicate that HsPEX5 binds directly to the SQL sequence at the free C terminus of HsPMP20 in the same manner as HsPEX5 binds to the typical PTS1 sequence.

**HsPMP20 Exhibits a Thiol-specific Antioxidant Activity**—In the Prodom data base (Prodom release 36), three yeast PMP20 proteins, CbPMP20A, CbPMP20B, and ScPMP20, are reported to contain the structural domain termed Prodom domain 210. The yeast PMP20 proteins share this domain with 72 other proteins, most of which are antioxidant proteins. Of the antioxidant proteins containing the domain 210, yeast PMP20 proteins showed a higher homology to thiol-specific antioxidant (TSA) proteins. Alignment analysis performed by DNASTAR showed that the amino acid sequence of domain 210 in HsPMP20 and in other TSA proteins was 56% identical and 76% similar. MmPMP20 also showed a high homology to these TSA proteins.

Therefore, the thiol-specific antioxidant activity of HsPMP20 was investigated. Antioxidant activity of HsPMP20 was analyzed by monitoring the ability of the protein to inhibit the inactivation of glutamine synthetase using an MCO system. HsPMP20 exerted, in a dose-dependent manner, an inhibitory effect on the inactivation of glutamine synthetase using a thiol-MCO system (DTT/Fe3+/O2) (Fig. 7A) but not using a nonthiol MCO system (ascorbate/Fe3+/O2) (Fig. 7B), suggesting that HsPMP20 does carry TSA activity. Furthermore, the protective activities of catalase and HsPMP20 on the inactivation of glutamine synthetase in the DTT/Fe3+/O2 system were compared. Catalase and HsPMP20 exerted protective effects in a dose-dependent manner (Fig. 7C). Both proteins could completely inhibit the inactivation of glutamine synthetase, and the concentration of proteins required to preserve 50% of the glutamine synthetase activity was 8 μg/ml for catalase and 40 μg/ml for HsPMP20. HsPMP20 removed H2O2 in the presence of DTT, suggesting that HsPMP20 acts as a TSA protein (Fig. 7D).

**DISCUSSION**

We have isolated two mammalian cDNAs encoding PMP20 and characterized the activity of the human protein. Compar-
ison of the deduced amino acid sequences of human and mouse PMP20s revealed 93% homology, indicating that PMP20s are highly conserved between these species, whereas their similarity to yeast PMP20 proteins was relatively limited. Human PMP20 mRNA was found in all human adult tissues examined. Interestingly, the expression of human PMP20 mRNA was very low in fetal brain, and increased post-natally in the adult brain.

Cell fractionation experiments reveal that HsPMP20 protein is present in the heavy membrane fraction corresponding to mitochondria, lysosomes, and peroxisomes, as well as in the cytoplasmic fraction. In these experiments, several control proteins such as HsPEX5, catalase, thiolase, EGF receptor, and NRP/B showed the expected associations with various subcellular fractions. Immunofluorescence studies confirmed the co-
localization of HsPMP20 with thiolase and catalase. We could not analyze the endogenous HsPMP20 localization, because we could not generate specific antibodies for HsPMP20. HsPMP20 was colocalized with thiolase and catalase, both markers for peroxisomal staining. It could be that the cytosolic localization observed is partly due to overexpression of the epitope-tagged HsPMP20.

Consistent with the localization of HsPMP20 to the peroxisomal matrix, this protein has a functional C-terminal PTS1 sequence, SQL. When HA-HsPMP20 and HsPEX5 were co-expressed, anti-HA monoclonal antibodies co-immunoprecipitated HsPEX5. In addition, anti-PEX5 antibodies co-immunoprecipitated HA-HsPMP20. Furthermore, Far Western analysis revealed that HsPEX5 protein could bind to both the purified HsPMP20 and GST-HsPMP20 but not to GST-HsPMP20ΔSQL. In addition, both GST-HsPMP20 and GST-HsPMP20Q161K could precipitate HsPEX5, and the amount of HsPEX5 bound to their C-terminal tripeptides, SQL and SKL, was similar. The mutant HsPMP20, lacking the C-terminal tripeptide, could not bind to HsPEX5. These results indicate that HsPMP20 and HsPEX5 can directly bind to each other in mammalian cells and that the tripeptide sequence SQL of HsPMP20 is solely required for binding to HsPEX5, the PTS1 receptor. This interaction between the PTS1 sequence of HsPMP20 and HsPEX5 would explain how HsPMP20 is targeted to peroxisomes. In view of the variants of the PTS1 sequence that function as PTSs, the ability of SQL to interact with HsPEX5 is not really surprising (33).

It has been shown that the yeast PMP20 proteins and numerous AhpC/TSA family proteins share the Prodom domain with various antioxidant proteins, especially with the TSA from various eukaryotes and prokaryotes. The amino acid alignment of the putative domain 210 of HsPMP20 exhibited homology to proteins of the AhpC/TSA family (56% identical, 76% similar), as do CbPMP20A, CbPMP20B, and ScPMP20. MmPMP20 also showed a homology to these thiol-specific antioxidant proteins. HsPMP20 exhibited antioxidant activity in the thiol-MCO system but not in the nonthiol MCO system, suggesting that HsPMP20 might function as an antioxidant enzyme containing functional cysteines. Peroxisomes contain several oxidases that use oxygen as an electron acceptor to oxidize organic substrates in the process of forming H₂O₂. Because peroxisomes lack an electron transport chain, electrons released during the oxidation of fatty acids are used to form H₂O₂, which is highly toxic to the cell. H₂O₂ is efficiently converted to H₂O within the peroxisomes by catalase. Abnormality in catalase import into peroxisomes is reported to lead to a severe neurological disorder. Like catalase, TSA owes its protective action to the removal of H₂O₂ (29). Both catalase and HsPMP20 exerted a protective effect, in a dose-dependent manner, in the thiol-MCO system where the H₂O₂ generated inactivates glutamine synthetase. In addition, we have shown that HsPMP20 re-
moves H$_2$O$_2$ by its thiol-peroxidase activity in the presence of DTT. Therefore, we propose that HsPMP20 is a novel member of the AhpC/ThsA family and is present in the peroxisomal matrix and possibly in the cytoplasm. HsPMP20 might play a role as a protector against oxidative stress in peroxisomes, as well as assist in the function of peroxisomal enzymes within the peroxisome. The source of thiols in peroxisomes, which are required for the TSA activity of HsPMP20, is still unknown. Further experiments should reveal the function of HsPMP20 as an antioxidant protein in peroxisomes.

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REFERENCES
1. Tolbert, N. E. (1981) Annu. Rev. Biochem. 50, 133–157
2. Van den Bosch, H., Schutgens, R. B. H., Wanders, R. J. A., and Tager, J. M. (1981) Annu. Rev. Biochem. 50, 133–157
3. Mannaerts, G. P., van Veldhoven, P. P., and Casteels, M. (1999) Cell Biochem. Biophys., in press
4. Gould, S. J., Keller, G. A., Shneider, M., Howell, S. H., Garrard, L. J., Goodman, J. M., Distel, B., Tabak, H., and Subramani, S. (1990) EMBO J. 9, 85–90
5. Swinkels, B. W., Gould, S. J., Bednar, A. G., Rachubinski, R. A., and Subramani, S. (1991) EMBO J. 10, 3255–3262
6. Lazarow, P., and Fujiki, Y. (1985) Annu. Rev. Cell Biol. 1, 489–530
7. Subramani, S. (1996) Curr. Opin. Cell Biol. 8, 513–518
8. Subramani, S. (1998) Physiol. Rev. 78, 171–188
9. Distel, B., Erdmann, R., Gould, S. J., Blobel, G., Crane, D. I., Cregg, J. M., Dott, G., Fujiki, Y., Goodman, J. M., Just, W. W., Kiel, J. A. K. W., Kunau, W. H., Lazarow, P. B., Mannaerts, G. P., Moser, H. W., Osumi, T., Rachubinski, R. A., Roscher, A., Subramani, S., Tabak, H. F., Tsukamoto, T., Valde, D., van der Klei, I., van Veldhoven, P. P., and Veenhuis, M. (1996) J. Cell Biol. 135, 1–3
10. Albertini, M., Rehling, P., Erdmann, R., Girzalsky, W., Kiel, J. A., Veenhuis, M., and Kunau, W. H. (1997) Cell 89, 83–92
11. Wiener, E. A. C., Nuttle, W. M., Bertolaet, B. L., Li, X., Francke, U., Wheeler, M. J., Anne, U. K., Johnson, K. R., and Subramani, S. (1995) J. Cell Biol. 130, 71–85
12. Dott, G., Braverman, N., Wong, C., Moser, A., Moser, H. W., Watkins, P., Valde, D., and Gould, S. J. (1995) Nat. Genet. 9, 115–125
13. Dott, G., and Gould, S. J. (1996) J. Cell Biol. 135, 1763–1774
14. Garrard, L. J., and Goodman, J. M. (1989) J. Biol. Chem. 264, 13929–13937
15. Goodman, J. M., Maher, J., Silver, P. A., Pacifico, A., and Sanders D. (1986) J. Biol. Chem. 261, 3464–3468
16. Goodman, J. M., Tropp, S. B., and Hwang H. (1990) J. Cell Sci. 97, 193–204
17. Veenhuis, M., and Goodman, J. M. (1990) J. Cell Sci. 96, 583–590
18. McNew, J. A., and Goodman, J. M. (1994) J. Cell Biol. 127, 1245–1257
19. Marshall, P. A., Krinkевич, Y. I., Lark, R. H., Dyer, J. M., Veenhuis, M., and Goodman, J. M. (1995) J. Cell Biol. 129, 345–356
20. Dyer, J. M., McNew, J. A., and Goodman, J. M. (1996) J. Cell Biol. 133, 269–280
21. Marshall, P. A., Dyer, J. M., Quick, M. E., and Goodman, J. M. (1996) J. Cell Biol. 135, 123–137
22. McCammon, M. T., McNew, J. A., Willy, P. J., and Goodman, J. M. (1994) J. Cell Biol. 124, 915–925
23. McNew, J. A., and Goodman, J. M. (1996) Trends Biochem. Sci. 21, 54–58
24. Antonenkov, V. D., Van Veldhoven, P. P., Waelkens, E., and Mannaerts, G. P. (1997) J. Biol. Chem. 272, 26023–26031
25. Fransen, M., Brees, C., Baumgart, E., Vanhooren, J. C. T., Baes, M., Mannaerts, G. P., and van Veldhoven, P. P. (1996) J. Biol. Chem. 271, 3703–3709
26. Akao, Y., Otsuki, Y., Kataoka, S., Itoh, Y., and Tsujimoto, Y. (1994) Cancer Res. 54, 2468–2471
27. Kim, T. A., Lim, J., Otsu, S., Baja, S., Rogers, R., Rivnay, B., Avraham, H., and Avraham, S. (1998) J. Cell Biol. 135, 555–566
28. Cha, M. K., and Kim, I. H. (1996) J. Cell Biol. 135, 567–575
29. Akao, Y., Otsuki, Y., Kataoka, S., Itoh, Y., and Tsujimoto, Y. (1994) Cancer Res. 54, 2468–2471
30. Cha, M. K., and Kim, I. H. (1996) J. Cell Biol. 135, 567–575
31. Kozak, M. (1984) Nucleic Acids Res. 12, 857–872
32. Elgersma, Y., van den Berg, A., van Roermund, C. W., van der Sluijs, P., Distel, B., and Tabak, H. F. (1996) J. Biol. Chem. 271, 26375–26382
33. Sheikh, F. G., Pahan, K., Khan, M., Barbosa, E., and Singh, I. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2961–2966