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Mutation in *PEX16* Is Causal in the Peroxisome-Deficient Zellweger Syndrome of Complementation Group D

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Summary

Peroxisome-biogenesis disorders (PBDs), including Zellweger syndrome (ZS), are autosomal recessive diseases caused by a deficiency in peroxisome assembly as well as by a malfunction of peroxisomes, among which >10 genotypes have been identified. We have isolated a human *PEX16* cDNA (*HsPEX16*) by performing an expressed-sequence-tag homology search on a human DNA database, by using yeast *PEX16* from *Yarrowia lipolytica* and then screening the human liver cDNA library. This cDNA encodes a peroxisomal protein (a peroxin Pex16p) made up of 336 amino acids. Among 13 peroxisome-deficiency complementation groups (CGs), *HsPEX16* expression morphologically and biochemically restored peroxisome biogenesis only in fibroblasts from a CG-D patient with ZS in Japan (the same group as CG-IX in the United States). Pex16p was localized to peroxisomes through expression study of epitope-tagged Pex16p. One patient (PBDD-01) possessed a homozygous, inactivating nonsense mutation, C→T at position 526 in a codon (CGA) for ^{176}Arg, that resulted in a termination codon (TGA). This implies that the C-terminal half is required for the biological function of Pex16p. PBDD-01–derived *PEX16* cDNA was defective in peroxisome-restoring activity when expressed in the patient's fibroblasts. These results demonstrate that mutation in *PEX16* is the genetic cause of CG-D PBDs.

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Introduction

Peroxisomes are present in a wide variety of eukaryotic cells, from yeast to human, and function in various metabolic pathways, including β-oxidation of very–long-chain fatty acids and the synthesis of ether lipids (van den Bosch et al. 1992). Peroxisomal proteins, including membrane proteins, are encoded by nuclear genes and translated on free polyribosomes in the cytosol (Lazarow and Fujiki 1985). Peroxisomes are formed by division of preexisting peroxisomes after the import of newly synthesized proteins (Lazarow and Fujiki 1985). Genetic analysis of peroxisome-biogenesis–defective mutants of yeast and mammalian cells has led to the identification of a number of protein factors, termed "peroxins," essential for peroxisome assembly (Distel et al. 1996; Fujiki 1997; Subramani 1997). The primary cause of peroxisome deficiency in a group of fatal genetic diseases, the peroxisome-biogenesis disorders (PBDs), which include Zellweger syndrome (ZS), neonatal adrenoleukodystrophy, and infantile Refsum disease, is a failure in peroxisome assembly (Shimozawa et al. 1992*a;* Lazarow and Moser 1995; Fujiki 1997; Subramani 1997). Genetic heterogeneity making up 14 complementation groups (CGs) has been identified in mammals through the use of fibroblasts from patients with PBDs, including rhizomelic chondrodysplasia punctata and peroxisome-deficient Chinese hamster ovary (CHO) cell mutants (Zoeller et al. 1989; Tsukamoto et al. 1990; Shimozawa et al. 1992*a;* Moser et al. 1995; Poulos et al. 1995; Okumoto et al. 1997; Tateishi et al. 1997; Tsukamoto et al. 1997; Kinoshita et al. 1998; Otera et al. 1998; Shimozawa et al. 1998*a*). Therefore, >14 genes are likely involved in mammalian peroxisome biogenesis. We have to date cloned *PEX2* (formerly "PAF-1"; Tsukamoto et al. 1991), *PEX6* (Tsukamoto et al. 1995), *PEX12* (Okumoto and Fujiki 1997; Okumoto et al. 1998*b*), and *PEX1* (Tamura et al. 1998) cDNAs by genetic phenotype-complementation assay of CHO cell mutants Z65, ZP92, ZP109, and ZP107, respectively. Several human orthologues of yeast peroxins have been isolated by means of expressed-sequence-tag (EST) analysis of the

human database, by use of yeast *PEX* genes (Subramani 1997; Abe et al. 1998; Okumoto et al. 1998*a*).

A nonsense mutation of the *PEX2* gene encoding a 35-kD peroxisome integral membrane protein was identified, for the first time, to be the primary cause of ZS in CG-F (the same group as CG-X in the United States and CG-5 in Europe; Shimozawa et al. 1992*b*). Dysfunction and mutations of *PEX5* encoding the PTS1 receptor were found in CG-II patients (Dodt et al. 1995; Wiemer et al. 1995). *PEX6* encoding a member of an ATPase family was shown to be responsible for ZS in CG-C (the same as CG-IV in the United States; Fukuda et al. 1996; Yahraus et al. 1996). *PEX12* and *PEX1* were recently shown to be mutated in PBD patients in CG-III (Chang et al. 1997; Okumoto and Fujiki 1997; Okumoto et al. 1998*b*) and CG-I (Portsteffen et al. 1997; Reuber et al. 1997; Tamura et al. 1998), respectively. More recently, mutation of *PEX10* was found to be the genetic cause of ZS in CG-B (the same as CG-VII in the United States; Okumoto et al. 1998*a*).

Herein we report how, by performing an EST homology search with *Yarrowia lipolytica PEX16*, we isolated human *PEX16* cDNA (*HsPEX16*) encoding a peroxin Pex16p. Peroxisome formation was reestablished in peroxisome-deficient fibroblasts from a CG-D patient (CG-IX in the United States) with PBD, by transfection of *HsPEX16*. We identified a homozygous mutation that inactivated *PEX16* in this patient.

Material and Methods

Cell Lines

Skin-fibroblast cell lines from patients, including several patients' fibroblasts transformed with SV40, were cultured in Dulbecco's modified Eagle's medium-high glucose supplemented with 10% fetal calf serum, as described elsewhere (Fukuda et al. 1996; Okumoto et al. 1998*b*). Fibroblasts from a CG-D patient (PBDD-01 [GM06231]) were purchased from the Human Genetic Mutant Cell Repository (Yajima et al. 1992). CHO cells were cultured as described elsewhere (Tsukamoto et al. 1990; Tsukamoto et al. 1991).

Cloning of Human PEX16

A GENETYX-MAC program (Software Development) was used to perform PCR on the human liver cDNA library in pCMVSPORT I (Gibco BRL; Tamura et al. 1998) with sense primer HsPEX16.FL (5′-ACGAATTCCGCAGGATGGAGAAGCTGC-3′ [the potential initiation codon is underlined]) and antisense primer HsPEX16.RL (5′-ATTGGATCCGGACAGCTCGTGCGAATCG-3′), containing nucleotide residues at positions 77–94 and 205–221, respectively, of the human EST AA357684 that showed good homology (38% identity) to *YlPEX16* (Eitzen et al. 1997). The PCR product was used as a probe to screen the human liver cDNA library (Tamura et al. 1998). Two positive clones, F6-7-18 and F6-7-39, were isolated from a subpool, F6-7, and their nucleotide sequences were determined; F6-7-39, made up of 1,171 bp, was termed "p-CMVSPORT•*HsPEX16*" and contained a 1,008-bp open reading frame (ORF) encoding a 336-amino-acid polypeptide.

Transfection of HsPEX16

pCMVSPORT•*HsPEX16* was transfected into PBD-patient fibroblasts by a Gene Pulser II electroporator (Bio-Rad) at a setting of 320 V/500 μF. Transfection to CHO cell mutants was performed with Lipofectamine (Gibco BRL) as recommended by the manufacturer (Okumoto et al. 1998*b*; Tamura et al. 1998).

Morphological Analysis

Peroxisomes in human fibroblasts and in CHO cells were visualized by indirect immunofluorescence microscopy, as described elsewhere (Shimozawa et al. 1992*a*). The antibodies used were raised, in rabbit, to human (Shimozawa et al. 1992*b*) and rat (Tsukamoto et al. 1990) catalase, to the 70-kD integral membrane protein of peroxisomes (PMP70) of rat (Tsukamoto et al. 1990; Kinoshita et al. 1998), and to the peroxisome-targeting signal type 1 (PTS1) peptide (Otera et al. 1998). Antigen-antibody complexes were detected by means of fluorescein isothiocyanate (FITC)–labeled sheep anti–rabbit immunoglobulin G antibody (Cappel), by a Carl Zeiss Axioskop FL microscope.

Mutation Analysis

Poly(A)$^+$ RNA was obtained from fibroblasts of a normal control and a CG-D patient (PBDD-01), with a QuickPrep mRNA purification kit (Pharmacia Biotech). Reverse transcription (RT)–PCR was performed with poly(A)$^+$ RNA (1 μg), oligo-dT primer (0.5 μg), and Superscript II reverse transcriptase (Gibco BRL) in 50 μl of the reaction mixture. To amplify the full length of the *PEX16* ORF, PCR was performed with a pair of human-specific PCR primers (50 pmol each), sense 16RTN (5′-GGAAGCTTGCCACCATGGAGAAGCTGCGGCTCCTGGGCCTCCGC-3′ [initiation codon underlined]) and antisense 16RTC (5′-CCCACTAGTGAATTCAGCCCCAACTGTAGAAGTAGATTTTCTG-3′ [termination codon underlined]), in 50 μl of the reaction mixture, which contained template DNA (⅛ aliquot of the RT product), 100 μM of dNTP, and 2.5 U of ExTaq DNA polymerase (Takara). Initial denaturation was done at 96°C for 45 s, followed by 25 cycles of denaturation at 96°C and annealing at 60°C, for 45 s each, and extension at 72°C for 90 s. PCR products

were cleaved with *Hin*dIII and *Spe*I, cloned into the *Hin*dIII/*Spe*I site of pBluescript II SK(−) (Gibco BRL), and sequenced. The patient's *PEX16* cDNA was digested with *Sal*I and *Not*I and cloned into the *Sal*I/*Not*I site of pCMVSPORT. Transfection of fibroblasts was done by electroporation.

Genomic DNA was prepared from cultured fibroblasts (Fukuda et al. 1996), with a QIAamp tissue kit (Qiagen). To investigate the zygosity of the *PEX16R176Ter* allele in patient PBDD-01, PCR amplification of the sequence between residues 471 and 697 was performed with the genomic DNA and a pair of *PEX16*-specific primers (50 pmol each): sense 16GF (5′-CAGCCCTGGCAACCATG-3′) and antisense 16GR (5′-GCAAGTGCAGCAGCGG-3′). Initial denaturation was performed at 96°C for 2 min, followed by 30 cycles of denaturation at 96°C, annealing at 55°C, and extension at 72°C, for 1 min each. PCR products were extracted and sequenced directly.

Expression of Epitope-Tagged Pex16p

Influenza virus hemagglutinin (HA)–epitope tagging of the C-terminus of Pex16p was performed by PCR with the forward primer HsPEX16F4 (5′-CCCTGCACTCCAGG-3′, with the nucleotide sequence at positions 548–561 and A starting in the initiation codon as 1) and the reverse primer HsPEX16HA (5′-CCCAAGCTTTCATGCATAATCGGGCACATCGTAGGGGTATGCATAATCGGGCACATCGTAGGGGTAGCTAGCGCCCCAACTGTAGAAGTAGATTTTC-3′; tandem HA-tag sequence underlined). The internal *Pst*I-*Hin*dIII fragment of the PCR product was cloned into the *Pst*I/*Hin*dIII site of pBluescript SK(-), to give pBluescript SK(-)•*HsPEX16-F4HA*. The *HsPEX16-HA* expression plasmid was constructed by introducing the *Pst*I-*Sal*I fragment of pBluescript SK(-)•*HsPEX16-F4HA* and the *Not*I-*Pst*I fragment of *HsPEX16* into the *Not*I/*Sal*I site of the pUcD2Hyg vector (Okumoto et al. 1998*b*). HA-tagged Pex16p expressed in cells was detected by means of mouse monoclonal antibody to HA (Boehringer Mannheim) and by FITC-labeled sheep anti–mouse IgG secondary antibody (Amersham).

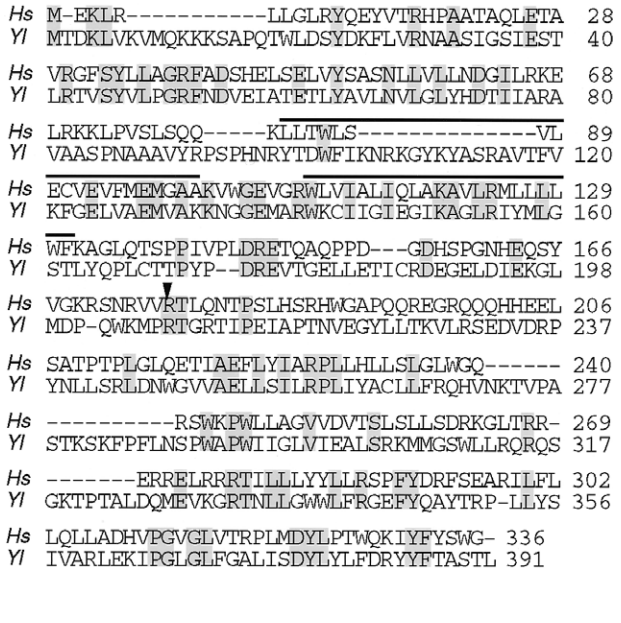

Figure 1 Deduced amino acid sequence of *HsPEX16* protein from CG-D ZS patient PBDD-01 (*Hs*), in comparison with that of Pex16p from *Y. lipolytica* (*Yl*). Broken lines (- - -) indicate a space. Amino acids that are identical in *HsPEX16* and *YlPEX16* proteins are shaded; hydrophobic segments are overlined. The arrowhead indicates the position of the mutation in the patient. The GenBank accession number for *HsPEX16* cDNA is AB016531.

Other Methods

Nucleotide sequencing was performed according to the dideoxy-chain termination method with a dye-terminator DNA sequence kit (Applied Biosystems). Alignment was effected with the CLUSTAL W program (EMBL).

Results and Discussion

Cloning of Human PEX16 *cDNA*

We identified several cDNA clones for the mammalian orthologue of *Y. lipolytica PEX16* (*YlPEX16;* Eitzen et al. 1997), including the human ESTs AA357684 and AA602247 and the mouse ESTs AA673100 and W97828, by the EST-homology search method—that is, BLAST search using the TBLASTN program (Altschul et al. 1990). We then isolated two positive clones by screening the human liver cDNA library with an AA357684-derived probe (see Material and Methods); one of the clones, F6-7-39, contained a 1,171-bp cDNA with an ORF encoding a 336-amino-acid protein of 38,642 daltons (fig. 1). Homology analysis suggested that this ORF was most likely to encode the human orthologue of *YlPEX16* (Eitzen et al. 1997). Therefore, we termed this cDNA "*HsPEX16*." The *HsPEX16* protein, *Hs*Pex16p, was shorter than *Yl*Pex16p by 55 amino acids in primary sequence, and the average amino acid identity to *Yl*Pex16p was 24%. Moreover, no potential N-glycosylation site was found in *Hs*Pex16p, but *Yl*Pex16p was reported to be N-glycosylated, presumably at position 272 (Titorenko and Rachubinski 1998).

PEX16 *Restores Peroxisome Assembly in CG-D Patient Fibroblasts*

Catalase in fibroblasts was visualized by immunostaining with the anti–human catalase antibody (Shi-

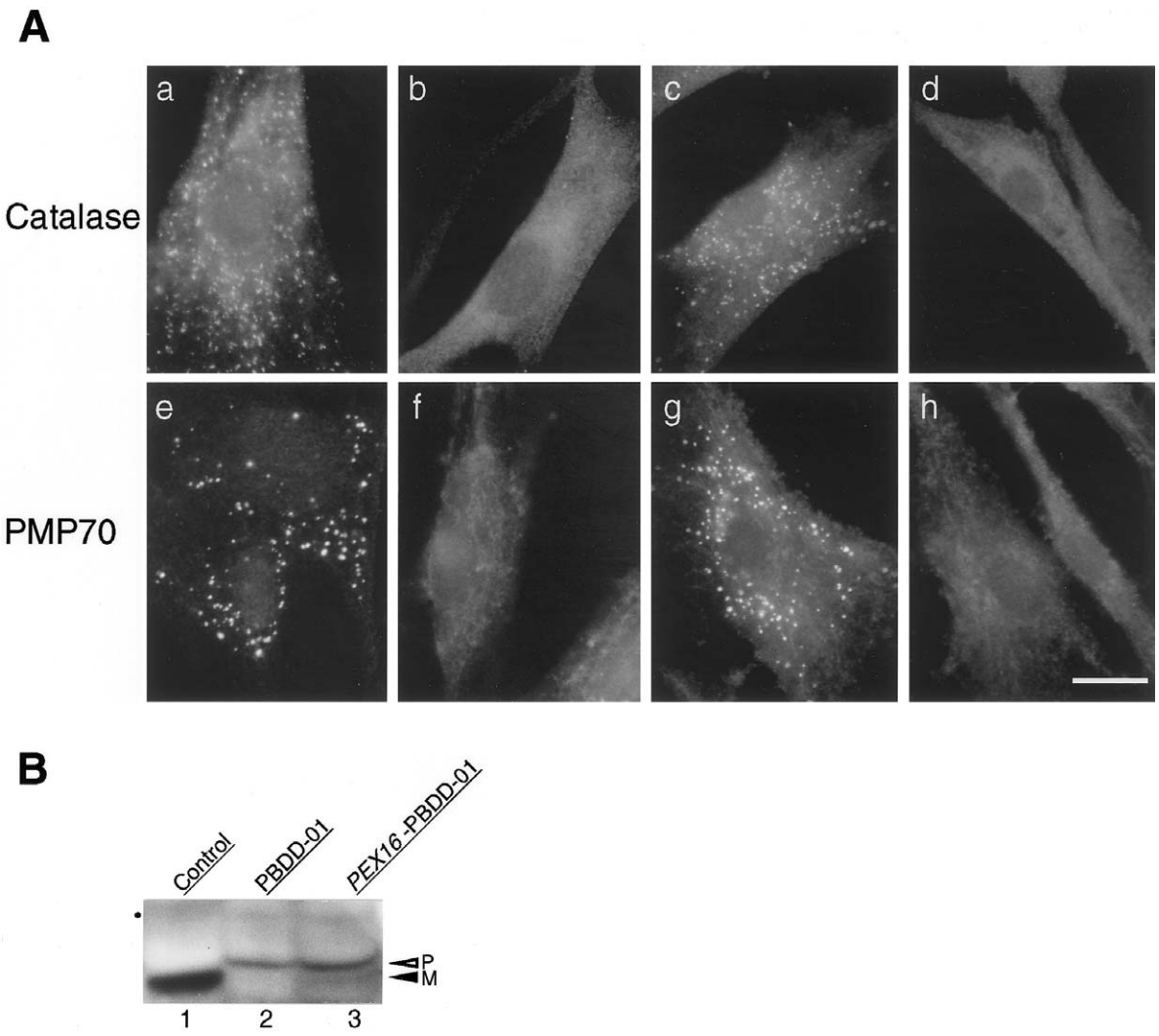

Figure 2 Complementation of peroxisomes in fibroblasts from a CG-D ZS patient. *A,* Transfection of *HsPEX16* into fibroblasts from a CG-D patient (PBDD-01) with ZS: fibroblasts from (*a*) a normal control, (*b–d* and *f–h*) PBDD-01, and (*e*) a CG-F patient with ZS (Shimozawa et al. 1992*b*). PBDB-01 fibroblasts were transfected with pCMVSPORT•*HsPEX16* (panels *c* and *g*) and PBDD-01–derived *PEX16*, *PEX16R176Ter* (panels *d* and *h*). Cells were stained with antibodies to catalase (panels *a–d*) and to PMP70 (panels *e–h*; magnification × 600 [bar=20 μm]). Note that peroxisomes were restored in *c* and *g* but not in *d* and *h*. *B,* Biogenesis of peroxisomal proteins. Cell lysates (2×10^5 cells) were subjected to SDS-PAGE and transferred to polyvinylidene difluoride membrane. Immunoblot analysis was done with rabbit anti–3-ketoacyl-CoA thiolase antibody (Tsukamoto et al. 1990). Lane 1, Normal control fibroblasts. Lane 2, PBDD-01 fibroblasts. Lane 3, PBDD-01 fibroblasts transiently transfected with *HsPEX16*. Unblackened and blackened arrowheads indicate a larger precursor (P) and mature form (M) of 3-ketoacyl-CoA thiolase, respectively; dot (·) indicates a nonspecific band.

mozawa et al. 1992*b*). Normal fibroblasts showed a punctate staining pattern typical of peroxisomes, whereas cells from a CG-D patient (PBDD-01) with ZS showed a diffuse, cytosolic staining indicative of peroxisome deficiency (fig. 2*A*, panels *a* and *b*). *HsPEX16* expression reestablished the assembly of peroxisomes in PBDD-01 fibroblasts, which were as numerous as those in normal cells (fig. 2*A*, panel *c*). Assessment by means of cell staining with antibodies to PTS1 and 3-ketoacyl-CoA thiolase, a PTS2 protein (data not shown), showed that peroxisomal protein import was also reestablished. Peroxisomal remnants, so-called ghosts (Santos et al. 1988), were discernible with anti-PMP70 antibody in fibroblasts from a *PEX2*-defective CG-F patient with ZS (Shimozawa et al. 1992*b*; fig. 2*A*, panel *e*), as reported for cells from peroxisome-deficient patients of all CGs so far examined (Santos et al. 1988; Wiemer et al. 1989; Santos et al. 1992; Wendland and Subramani 1993). In contrast, consistent with the recent findings by Shimozawa et al. (1998 [in this issue]), peroxisomal ghosts

were not detected in PBDD-01 fibroblasts, apparently indicating a defect in peroxisome membrane biogenesis (fig. 2*A*, panel *f*). On transfection of *HsPEX16*, PMP70-positive peroxisomes were formed (fig. 2*A*, panel *g*), but the patient PBDD-01–derived *PEX16* could not complement peroxisome assembly (fig. 2*A*, panels *d* and *h*; see below). Taken together, these results demonstrated that Pex16p morphologically complemented peroxisome biogenesis in PBDD-01 fibroblasts.

We also investigated the biogenesis of peroxisomal proteins in CG-D fibroblasts. Peroxisomal 3-ketoacyl-CoA thiolase is synthesized as a larger, 44-kD precursor with an amino terminal, cleavable PTS2 (Osumi et al. 1991; Swinkels et al. 1991), and is processed to its final size, 41 kD, in peroxisomes (Tsukamoto et al. 1990). In normal fibroblasts, only mature thiolase was detected (fig. 2*B*, lane 1), thereby demonstrating rapid processing of the precursor form. In PBDD-01 cells, only the larger precursor was found (lane 2), implying a defect of import and processing activity. When PBDD-01 fibroblasts were transiently transfected with *HsPEX16*, both mature and unprocessed forms of thiolase were seen (fig. 2*B*, lane 3). The smaller amount of mature thiolase, compared with that of the precursor, possibly reflects the efficiency of complementation—that is, ~10%, at 3 d posttransfection (see table 1). Thus, these results demonstrate that *HsPEX16* can complement the impaired biogenesis of peroxisomal proteins in PBDD-01 cells.

HsPEX16 was also introduced into fibroblasts from four other PBD CGs: A, B, and G of Gifu University and group VI of the Kennedy-Krieger Institute. As expected, no fibroblasts of these CGs were complemented (table 1). *HsPEX16* expression could not restore peroxisomes in eight CGs of peroxisome-deficient CHO cell mutants, of which five represented PBD CGs (Tsukamoto et al. 1990; Shimozawa et al. 1992*a*; Okumoto et al. 1997; Tateishi et al. 1997; Kinoshita et al. 1998; Otera et al. 1998; table 1). Taken together, these results confirmed that Pex16p is the peroxisome-biogenesis factor for the PBD of CG-D.

Mutation Analysis of CG-D Patient PEX16

To determine the dysfunction of *PEX16* in patient PBDD-01, we isolated *PEX16* cDNA from fibroblasts of PBDD-01 and a normal control, by RT-PCR. Subsequent sequencing of cDNA clones from the patient indicated a C→T point mutation at nucleotide position 526 (with the A of the initiating ATG being 1) in a codon (CGA) for Arg, resulting in a premature termination at

Table 1

Complementation Analysis of Patient Fibroblasts and CHO Cell Mutants by *HsPEX16*

Source of Patient Fibroblasts	Peroxisome Positive	CHO Mutant	Peroxisome-Positive Cells	Gene
CG-D (PBDD-01) [CG-IX]	19/200			*PEX16*
CG-A [CG-VIII]	…			
CG-B [CG-VII]	…			*PEX10*
CG-(C) [CG-IV]		ZP92	…	*PEX6*
CG-(E) [CG-I]		ZP107	…	*PEX1*
CG-(F) [CG-X]		Z65	…	*PEX2*
[CG-II]		ZP105	…	*PEX5*
[CG-III]		ZP109	…	*PEX12*
[CG-VI]	…			
CG-G	…			
		ZP110		…
		ZP114		…
		ZP119		…

NOTE.—Patient fibroblasts from five CGs of PBDs (Fujiki 1997)—namely, CGs D (patient PBDD-01), A, B, and G of Gifu University, Gifu, Japan, and CG-VI of Kennedy-Krieger Institute, Baltimore—and peroxisome-deficient CHO mutants of eight CGs (Fujiki 1997; Tateishi et al. 1997; Kinoshita et al. 1998)—including *PEX6*-defective ZP92 (Shimozawa et al. 1992*a;* Tsukamoto et al. 1995), *PEX1*-impaired ZP107 (Okumoto et al. 1997; Tamura et al. 1998), *PEX2*-deficient Z65 (Tsukamoto et al. 1991; Shimozawa et al. 1992*b;* Tsukamoto et al. 1994), *PEX5*-defective ZP105 (Okumoto et al. 1997; Otera et al. 1998), *PEX12*-deficient ZP109 (Okumoto et al. 1997; Okumoto and Fujiki 1997; Okumoto et al. 1998*b*), and recently isolated ZP119 (Kinoshita et al. 1998)—were transfected with pCMVSPORT•*HsPEX16* and examined for peroxisome assembly by immunostaining with antisera to human catalase (in the case of fibroblasts) and rat catalase (in the case of CHO mutants) at 3 d posttransfection. CGs in parentheses were not used in this experiment. CGs in brackets indicate U.S. nomenclature; ellipses (…) indicate not complemented.

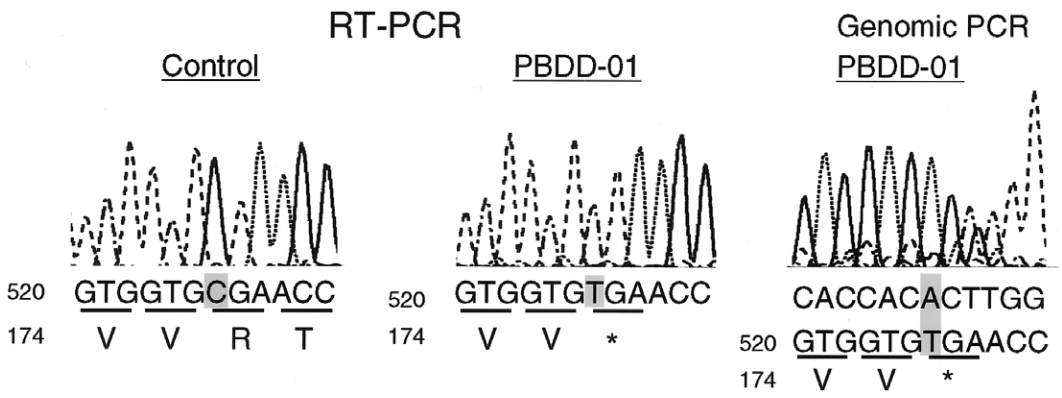

Figure 3 Mutation analysis of *PEX16* from a CG-D ZS patient. Partial nucleotide sequence and deduced amino acid sequence of *PEX16* cDNA isolated from a normal control (*left*) and from ZS patient PBDD-01 (*middle*) are shown. A point mutation at nucleotide residue 526 (*shaded region*) caused a nonsense mutation in PBDD-01 *PEX16* (*middle*). PCR was also done for DNA from PBDD-01 fibroblasts (*right*). The nucleotide sequence was determined for the antisense strand (*above*); its complementary sense sequence is also shown (*below*).

^{176}Arg (fig. 3, *left* and *middle*). Of 20 cDNA clones isolated, termed "*PEX16R176Ter,*" all showed the same mutation, thereby suggesting a homozygous mutation. To determine the zygosity of a *PEX16R176Ter* mutant allele, genomic PCR was done to amplify the sequence corresponding to nucleotide residues 471–697 in the *PEX16* ORF. Only a single type of nucleotide sequence giving rise to the R176Ter mutation was identified in the PCR products (fig. 3, *right*), and a 135-bp intron was found between residues 541 and 542 (data not shown). The results were interpreted to mean that ZS patient PBDD-01 was homozygous for the R176Ter mutation. This mutation, as assessed by back transfection of *PEX16R176Ter* to PBDD-01 fibroblasts, inactivated the function of *PEX16*, which resulted in no formation of either peroxisomes or peroxisomal membrane vesicles (fig. 2*A*, panels *d* and *h*). These findings suggest the importance of the C-terminal half of Pex16p in its biological activity. It is equally plausible that the truncation may affect the stability of the expressed Pex16p.

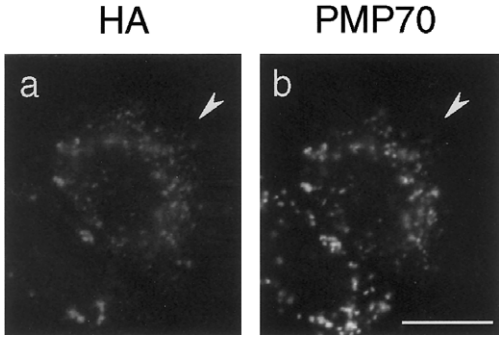

Figure 4 Intracellular localization of Pex16p. Human Pex16p tagged with HA at its C-terminus was expressed in wild-type CHO-K1 cells. Cells were stained with mouse anti-HA antibody (*a*) and rabbit anti-PMP70 antibody (*b*). Rabbit IgG was detected with Texas Red–labeled goat anti–rabbit IgG antibody (Leinco Technologies). Arrowheads indicate a Pex16p-HA–expressing cell. Note that punctate structures, peroxisomes, are superposable in panels *a* and *b*. Large spots are apparently unwashed lipofectamine or its debris (magnification ×630 [bar= 20 μm]).

Intracellular Localization of Pex16p

Subcellular localization of Pex16p was determined by immunofluorescence microscopy following ectopic expression of Pex16p tagged with the HA epitope at its C-terminus. *HsPEX16HA* expression restored peroxisome assembly in PBDD-01 fibroblasts, indicating that C-terminal tagging did not interfere with Pex16p function, although Pex16p-HA protein was not detected by use of anti-HA antibody, presumably because of a low level of expression (data not shown). In *HsPEX16HA*–transfected CHO-K1 cells, Pex16p was detected in a punctate staining pattern (fig. 4*A*, panel *a*). The pattern was superposable on that obtained with anti-PMP70 antibody (fig. 4*A*, panel *b*), thereby demonstrating that Pex16p-HA was localized to peroxisomes. *Yarrowia lipolytica* Pex16p was shown to be a peroxisomal peripheral membrane protein with four putative hydrophobic domains (Eitzen et al. 1997). It would be intriguing to investigate whether human Pex16p, like the yeast protein, shows intraperoxisomal localization.

Collectively, the data in the present study demonstrate that dysfunction of *PEX16* is responsible for peroxisome deficiency in CG-D patients with PBDs. Peroxisomal ghosts have been reported in fibroblasts from peroxisome-deficient patients (Santos et al. 1988; Wiemer et al. 1989; Santos et al. 1992; Wendland and Subramani 1993). In contrast, CG-D fibroblasts are apparently defective in peroxisomal membrane biogenesis, as was the

case for a CHO mutant ZP119 that we recently characterized (Kinoshita et al. 1998). Pex16p expression restores peroxisome assembly in CG-D cells, evidently indicating that Pex16p is involved in peroxisome assembly, presumably at the stage of peroxisomal membrane vesicle formation. Therefore, Pex16p is the first peroxin that complements PBD cells morphologically devoid of peroxisomal remnants. These results may also suggest that peroxisomes can form de novo and do not have to arise from preexisting peroxisomes.

Six mammalian peroxin genes involved in peroxisome assembly have been isolated either by homology search of the human EST database or by genetic functional complementation of CHO cell mutants: *PEX1* for CG-E in Japan (CG-I in the United States; Portsteffen et al. 1997; Reuber et al. 1997; Tamura et al. 1998); *PEX2* for CG-F in Japan (Shimozawa et al. 1992*b*); *PEX5* for CG-II (Dodt et al. 1995; Wiemer et al. 1995; Otera et al. 1998); *PEX6* for CG-C in Japan (Tsukamoto et al. 1995; Fukuda et al. 1996; Yahraus et al. 1996); *PEX10* for CG-B in Japan (Okumoto et al. 1998*a*); and *PEX12* for CG-III (Chang et al. 1997; Okumoto and Fujiki 1997; Okumoto et al. 1998*b;* see also table 1). Thus, *PEX16* is the seventh gene identified as being responsible for peroxisome-deficiency diseases. *PEX7* encoding the PTS2-receptor was mutated in patients with rhizomelic chondrodysplasia punctata, in which morphologically normal peroxisomes are present, but those peroxisomes are defective in the import of PTS2 proteins (Braverman et al. 1997; Motley et al. 1997; Purdue et al. 1997). It is possible that Pex16p functions, through interaction with other *PEX* proteins, as the peroxisome-biogenesis factor in the peroxisomal protein-import processes (Distel et al. 1996; Fujiki 1997; Subramani 1997; Okumoto et al. 1998*a,* 1998*b;* Tamura et al. 1998). Pex16p may also be involved in the proliferation of peroxisomes, as noted in *Y. lipolytica* (Eitzen et al. 1997).

Acknowledgments

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Electronic-Database Information

Accession number and URL for data in this article are as follows:

GenBank, http://www.ncbi.nih.gov./Web/GenBank (for human *PEX16* cDNA sequence [accession number AB016531])

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