# Cloning and characterization of the gene encoding the human peroxisomal assembly protein Pex3p

Stefan Kammerer[a,*], Andreas Holzinger[a], Ulrich Welsch[b], Adelbert A. Roscher[a]

[a] *Dr. von Hauner Children's Hospital, Department of Clinical Chemistry and Biochemistry, Laboratory of Molecular Biology, Ludwig-Maximilian-University of Munich, Lindwurmstrasse 4, 80337 Munich, Germany*
[b] *Department of Anatomy, Ludwig-Maximilian-University of Munich, Pettenkoferstrasse 11, 80336 Munich, Germany*

Received 10 February 1998; revised version received 30 April 1998

**Abstract Proteins essential for the assembly of functional peroxisomes are designated peroxins and are encoded by *PEX* genes. In yeast, Pex3p was previously identified as a peroxisomal integral membrane protein indispensable for peroxisome biogenesis and integrity. Here we report the cloning of the orthologous human *PEX3* gene. It encodes a polypeptide of 373 amino acids (42 kDa) and is expressed in all tissues examined. As shown by transfection of epitope tagged constructs and immunofluorescence analysis, human Pex3p is localized at the peroxisome. The N-terminal 40 amino acids were revealed to be sufficient to target a GFP reporter protein to the peroxisome. A positively charged five amino acid sequence within this N-terminal region is highly conserved from yeast to human Pex3p. Overexpression of human Pex3p leads to proliferation of ER membranes in COS7 cells. Since disruption of human peroxins has been shown to result in peroxisomal biogenesis disorders, *PEX3* is another candidate gene being involved in this disease group.**



*Key words:* Peroxisomal assembly; Peroxin; Yeast ortholog; Ubiquitous expression; Peroxisomal ghost; Targeting signal

## 1. Introduction

Peroxisomes are ubiquitous organelles in eukaryotes involved in a variety of metabolic pathways [1]. Studies in yeast peroxisomal mutants have been instrumental in investigating the evolutionarily highly conserved mechanisms of peroxisome biogenesis including peroxisome proliferation, membrane biogenesis and peroxisomal matrix protein import via specific targeting signals (PTS) and their corresponding receptors. Proteins implicated in these processes have recently been designated peroxins and are encoded by *PEX* genes [2]. The actual list of peroxins comprises at least 19 proteins. In humans, defects of peroxins cause the heterogeneous group of peroxisomal biogenesis disorders (PBDs) including Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum's disease and classical rhizomelic chondrodysplasia punctata [3,4]. Based on somatic cell fusion complementation analysis with human fibroblasts and/or Chinese hamster ovary cell lines at least 13 genes may contribute to the pathogenesis of mammalian PBDs [5,6]. Six of the human genes underlying one of these complementation groups have been certainly identified to date: *PEX5* [7] and *PEX7* [8–10], which encode the receptors for the peroxisomal targeting signals PTS1 and PTS2, respectively; *PEX1*, mutations therein causing the majority of human PBDs [11,12], and *PEX6* [13], which both encode AAA ATPases; finally *PEX2* [14] and *PEX12* [15], which encode zinc-binding integral membrane proteins. All of these proteins are engaged in the import of peroxisomal proteins and their deficiency leads to cells that lack functional peroxisomes but morphologically show residual peroxisomal structures, described as peroxisomal ghosts.

Yeast cells deficient in the farnesylated protein Pex19p seem to have not even peroxisomal ghosts [16]. The same phenomenon was described for yeast Pex3p [16–19]. This has led to the suggestion that these two proteins might be implicated in the early stages of peroxisome assembly and renewed questions with regard to the origin of peroxisomes. The current opinion is that they are derived from pre-existing peroxisomes by growth and division [20]. However, *pex3*Δ and *pex19*Δ yeast mutants, while not having any recognizable peroxisomal remnants, do have the capacity to restore intact peroxisomes during complementation with the corresponding genes. These results may support the hypothesis of Waterham and coworkers [21] that peroxisomes are not necessarily derived from pre-existing organelles. Data on the Pex15 protein of *Saccharomyces cerevisiae* are also consistent with potential de novo synthesis of peroxisomes [22]. The proliferation of endoplasmic reticulum (ER) derived membranes by overexpression of Pex15p and post-translational modifications that are thought to be restricted to the ER revive the idea that new peroxisomes may form by budding from the ER. Pex3p from *Hansenula polymorpha* also seems to be targeted via the ER since fusion proteins containing the N-terminal 16 amino acids of HpPex3p were detected at probably ER derived membranous structures [18].

In yeast, Pex3p has been shown to interact with Pex19p in vivo [16]. We have recently cloned and characterized the human ortholog of Pex19p, designated HsPXF [23,24], a farnesylated protein loosely attached to the peroxisomal membrane. In this study we report the identification of the human ortholog of the yeast Pex3p, which is a ubiquitously expressed integral membrane protein of the peroxisome.

*Corresponding author. Fax: (49) (89) 5160 4192.
E-mail: kammerer@kk-i.med.uni-muenchen.de

*Abbreviations:* (r)ER, (rough) endoplasmic reticulum; EST, expressed sequence tag; Hp, *Hansenula polymorpha*; Pex, peroxin; PBD, peroxisome biogenesis disorder; Pp, *Pichia pastoris*; PTS, peroxisomal targeting signal(s); RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription-polymerase chain reaction; Sc, *Saccharomyces cerevisiae*; TM, transmembrane

The sequence from this article has been deposited in the GenBank/EMBL data libraries under accession number AJ001625. All accession numbers given in parentheses throughout the text are derived from these databases.


*PII* S0014-5793(98)00557-2

## 2. Materials and methods

### 2.1. Cloning human PEX3 full-length cDNA

We probed the dbEST database [25] with the Pas2 protein sequence from *Pichia pastoris* [19]. The identified human EST clone 176494 (AA305508) was obtained from the TIGR/ATCC collection and the entire sequence determined by fluorescent dye terminator sequencing on an ABI 377 sequencer. The very 5′ cDNA end was obtained by RT-PCR using 4 μg of total liver RNA, a human *PEX3*-specific reverse primer (375R, 5′ CTT ATT ATC TTC AGA TCC TCC 3′) and a first-strand cDNA synthesis kit (Pharmacia). PCR amplification was performed with 1 μl of cDNA, a nested reverse primer from the coding region (232R, 5′ GAT ATT GTC GTC GTG CTT GG 3′) and a forward primer deduced from the sequence of the EST clone AA471379 (5′F1, 5′ GTC TAA TGA AAA GGC ACT CG 3′). Both reverse primers were chosen such that an intron would be included in a PCR product from genomic DNA. The PCR product was directly sequenced. The usage of an alternative poly(A) site in the 3′ untranslated region was confirmed by 3′RACE-PCR using a Marathon PCR-ready liver cDNA (Clontech) and sequence analysis of several subclones.

### 2.2. Computational analysis

Database searches for homologous protein or EST sequences were performed using the blastp or tblastn program, respectively (NCBI, National Center of Biotechnology, Washington, DC, USA). The ClustalW program [31] from the Baylor College of Medicine was used for the generation of protein alignments. Protein similarity scores were determined using the program Align from EERIE, Nîmes, France. Transmembrane prediction graphics were produced using the TMpred server of the Bioinformatics group at ISREC, Epalinges, Switzerland. This algorithm is based on the statistical analysis of TMbase, a database of naturally occurring transmembrane proteins [32].

### 2.3. Northern blot analysis

A human multiple tissue Northern blot containing 2 μg of poly(A)$^+$ RNA per lane was purchased from Clontech. The 1119 bp probe was produced by PCR from EST clone 176494 plasmid DNA with primers Pex3F (5′ CCA CCA TGC TGA GGT CTG TAT GGA AT 3′) and Pex3R (5′ TAC CCG GGT TTC TCC AGT TGC TGA GGG 3′) spanning the complete coding region and radiolabeled by random priming with [α-$^{32}$P]dATP. Hybridization was performed by a modified method of Church and Gilbert [33] as described elsewhere [23]. The blot was then exposed to an X-ray film for 12 days.

### 2.4. Expression of epitope tagged Pex3p

For the construction of a *PEX3-myc* vector, the complete coding region of the human *PEX3* gene was amplified by PCR using Vent DNA Polymerase (New England Biolabs) and 200 ng plasmid DNA of the EST clone 176494. The gel purified fragment was blunt end ligated into an *Eco*RV digested pcDNA3.1/Myc-His plasmid (Invitrogen) in front of the myc encoding region. Using also the EST clone 176494 as template, the first 120 bp of the coding region encoding the N-terminal 40 amino acids of Pex3p were amplified with Vent DNA polymerase and primers containing appropriate restriction sites for directional cloning. The PCR product was digested with *Bam*HI and *Sal*I and cloned to the 5′ end of the coding region of the green fluorescent protein (GFP) utilizing the vector pEGFP-N1 (Clontech). All clones were checked by PCR for insert orientation and sequenced.

Purified plasmid DNA (2 μg, Maxi-Prep Kit, Qiagen) was used for transfection of COS7 cells. For this purpose, $2\times10^5$ COS7 cells were seeded onto coverslips in six-well culture plates in DMEM containing 10% fetal calf serum and then grown to about 70% confluence. Cells were transfected with plasmid DNA and lipofectamine following the manufacturer's instructions (Gibco-BRL). For establishing stable Pex3-myc expressing integrants, transfected COS7 cells were selected several times with 500 μg/ml geneticin (Boehringer Mannheim).

### 2.5. Immunofluorescence and electron microscopic analysis

Indirect immunofluorescence staining was started 48 h after transfection by rinsing the cells twice with PBS. Cells were then fixed with 4% formaldehyde in PBS for 20 min and washed once with PBS. After permeabilization in 1% Triton X-100 in PBS or 25 μg/ml digitonin in PBS for 5 min, coverslips were washed three times with PBS and co-incubated for 1 h with a 1:100 dilution of one or more of the following antibodies: monoclonal anti-myc antibodies (Invitrogen), rabbit anti-human erythrocyte catalase antibodies (Biodesign), rabbit anti-human PMP70 antibodies (kindly provided by Wilhelm Just, University of Heidelberg, Germany) and rabbit anti-GFP IgG (Clontech). Coverslips were then washed 10 times with PBS and incubated for 30 min with secondary antibodies: rhodamine conjugated goat anti-mouse IgG, fluorescein conjugated goat anti-rabbit IgG and/or rhodamine conjugated goat anti-rabbit IgG (all Jackson ImmunoResearch Laboratories). After 10 washing steps with PBS, coverslips were mounted with Vectashield mounting medium (Vector) and analyzed microscopically.

For electron microscopic analysis, stable integrants and untransfected cells were cultivated in 75 cm$^2$ flasks to about 70% confluence, detached with trypsin and washed twice with PBS. After fixation with 2.5% glutaraldehyde, cells were postfixed with 1% osmium tetroxide. After that they were dehydrated in ethanol and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and subsequently examined with a Philips CM 10 electron microscope.

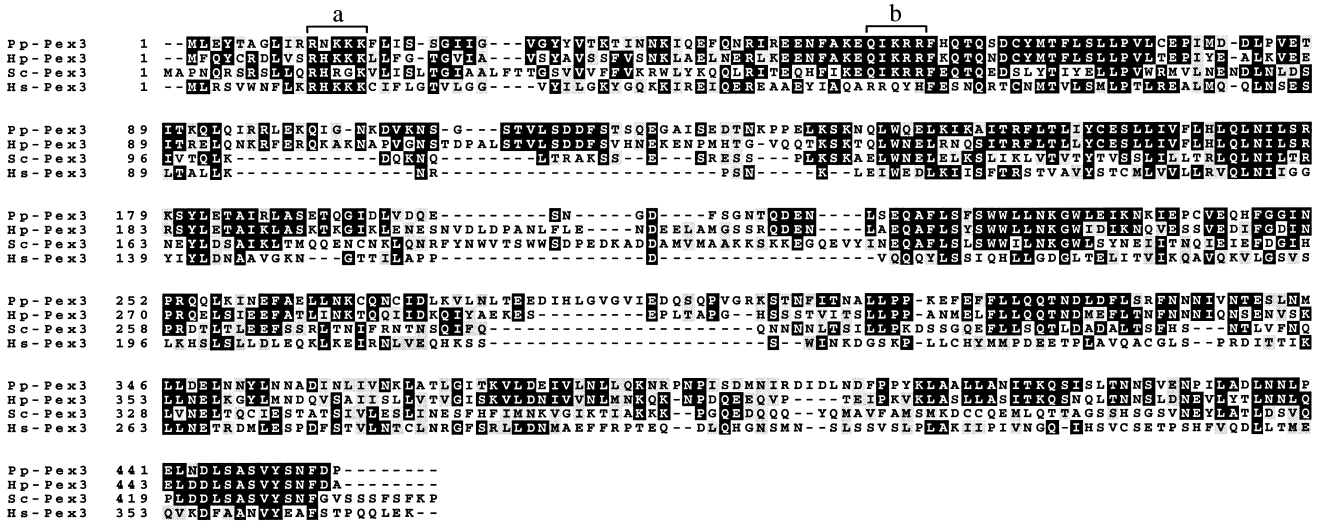


Fig. 1. Alignment of Pex3p from various species. The yeast Pex3 proteins from *H. polymorpha* (HpPex3p), *P. pastoris* (PpPex3p) and *S. cerevisiae* (ScPex3p) were aligned with the presumed human ortholog HsPex3p using the ClustalW program. Amino acids identical in two or more proteins are boxed in black, conserved residues are boxed in gray. Putative targeting motifs discussed in the text are indicated by overlines (a and b).

## 3. Results

### 3.1. Identification of the full-length human PEX3 cDNA

The dbEST database [25] was screened with the amino acid sequence of the *Pichia pastoris* Pex3 (formerly: Pas2) protein [19] as probe for homologous human cDNA sequences. We identified several expressed sequence tags (EST), one of which (EST176494, AA305508) seemed to contain the complete coding region of the human ortholog. This EST clone from a human colon carcinoma cell line was sequenced and revealed a 5′ untranslated sequence of 118 bp, followed by an open reading frame of 1119 bp (373 amino acids) and a 3′ untranslated sequence of 780 bp. A consensus poly(A) signal was identified 16 bp upstream of the poly(A) at position +2179 relative to the transcription start site. Two other human EST clones (T62150, C00647) showed a 3′ untranslated sequence of only 136 bp, indicating an alternative poly(A) addition in this transcript variant. 3′RACE-PCR was used to verify these transcripts. By sequence analysis we identified a consensus poly(A) signal at position +1532, 19–28 bp upstream of the poly(A) tail. The EST clone including the longest 5′ untranslated sequence (AA471379) extended 294 bp upstream of the initiation codon. The existence of this sequence at the RNA level was confirmed by RT-PCR from specifically primed liver cDNA and sequence analysis of the generated PCR product thereby excluding a cloning artifact in the EST library.

A database search using the 373 amino acid sequence showed a similarity of about 30% identical residues to a *Caenorhabditis elegans* open reading frame (U56965) from a cosmid clone of chromosome III [26]. This putative protein, however, terminates after the N-terminal 122 amino acids perhaps as a consequence of a wrong exon/intron interpretation. High similarities were also found with several mouse ESTs (AA030379, AA510315, AA153915, AA475553) and an EST from a *Drosophila melanogaster* embryo (AA264129). The murine sequences available from the database seem to encode the central 182 and the C-terminal 31 amino acids of the protein and show 90.2% total amino acid identity with the human protein. The homologous *D. melanogaster* EST sequence from the database apparently encodes the N-terminal 106 amino acids which show 36.8% identical residues in comparison with the human protein.

### 3.2. Sequence and structural comparison with yeast Pex3 proteins

An alignment of the 373 amino acid sequence with Pex3

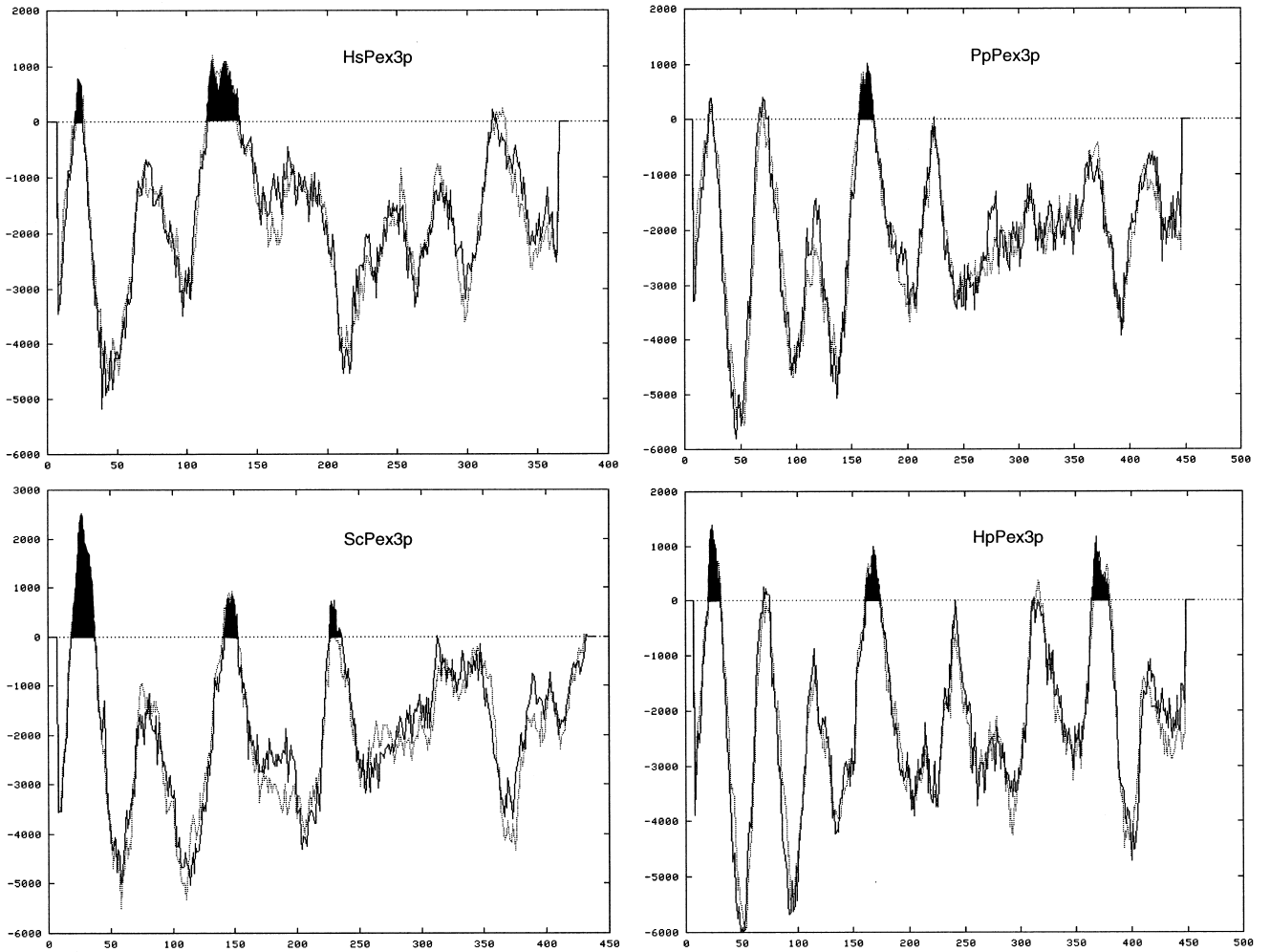


Fig. 2. Transmembrane (TM) prediction for yeast and human Pex3 proteins. TM prediction graphics were produced using the TMpred server. The length of the hydrophobic part of TM helices was set to encompass 17–33 amino acids. The predicted transmembrane segments are highlighted.

proteins of several yeast species is shown in Fig. 1. A pairwise alignment revealed an overall amino acid identity of 23.1% to the *Pichia pastoris* (455 amino acids), 20.2% to the *Hansenula polymorpha* (457 amino acids), and 19.7% to the *Saccharomyces cerevisiae* (441 amino acids) protein.

The prediction of transmembrane (TM) segments proposed two strong TM helices for the human Pex3p (Fig. 2). For the yeast proteins, the TMpred program also predicted one or more TM helices qualifying them as integral membrane proteins as published previously [17–19]. The TMpred profile for the human Pex3p is very similar to the yeast profiles showing the two predicted transmembrane regions in the N-terminal half of the protein (highlighted in Fig. 2). Only HpPex3p exhibits another potential TM segment in the C-terminal half.

### 3.3. Expression of the PEX3 gene in human tissues

Using a cDNA probe containing the complete coding region, Northern blot hybridization of mRNA isolated from multiple human tissues was performed (Fig. 3). Fragments of 1.6 and 2.3 kb were detected. The 2.3 kb transcript seems to be predominant, but both transcripts are present in all tissues examined. In addition to the tissues analyzed by Northern blot, human *PEX3* is obviously expressed in cells and tissues where EST clones were derived from: a colon carcinoma cell line (AA305508), a parathyroid tumor (W19665) and promyeloblasts (AA471379).

### 3.4. Subcellular localization and targeting of human epitope tagged Pex3p

In order to determine the subcellular localization of the human Pex3 protein, epitope tagged Pex3p was overexpressed in COS7 cells and detected by indirect immunofluorescence (Fig. 4). In the Pex3p-myc fusion protein, a myc epitope of 10 amino acids (followed by six histidine residues) is attached to the C-terminus of Pex3p. Overexpressing COS7 cells were permeabilized with Triton X-100 and co-stained with c-myc antibodies and antibodies to catalase as a peroxisomal marker protein. The c-myc antibodies created a punctate pattern typical of peroxisomal distribution (Fig. 4A). The same pattern was seen using antibodies to catalase (Fig. 4B). In digitonin treated cells in which the peroxisomal membranes are not permeabilized, staining with c-myc antibodies also produced a punctate pattern characteristic of peroxisomes (Fig. 4C), while the antibody to the peroxisomal matrix protein catalase did not create such a pattern (Fig. 4D). In contrast, polyclonal antibodies to the peroxisomal membrane protein PMP70 which was used as marker for unpermeabilized peroxisomes detected the same peroxisomal structures as the myc antibodies in digitonin treated cells (Fig. 4E,F). No peroxisome-like structures were detected with any of these primary antibodies or with secondary antibodies alone (not shown). These results demonstrate that human Pex3p is localized at the peroxisome and that the C-terminus of the fusion protein is most likely exposed to the cytosol.

In targeting studies the N-terminus of Pex3p was analyzed for its capacity to direct a reporter protein to the peroxisome. For this purpose the N-terminal 40 amino acids fused to the N-terminus of the green fluorescent protein (GFP) were expressed in COS7 cells (Pex[1–40]GFP). Fluorescence microscopic analysis revealed a punctate pattern (Fig. 5A,C) identical to the Pex3p-myc protein. An identical picture was achieved by staining with anti-catalase antibodies in Triton X-100 permeabilized cells (Fig. 5B). By using anti-GFP antibodies it was shown that the GFP portion of Pex[1–40]GFP is localized on the cytosolic surface of the peroxisome since it was detectable in digitonin treated cells (Fig. 5D). COS7 cells expressing GFP from the EGFP-N vector without insert did not show a punctate pattern but only green fluorescent background (Fig. 5F).

### 3.5. Subcellular morphology of Pex3p overexpressing cells

The effect of overexpression was examined by investigating stably transfected COS7 cells expressing Pex3p-myc fusion protein by electron microscopy (Fig. 6). The comparison with untransfected cells revealed a morphological change of the rough endoplasmic reticulum (rER) in Pex3p overexpressing cells. In most sections analyzed, untransfected cells showed only low presence of ER membranes predominantly in the vicinity of the nucleus while no ER was visible in the bulk of cytoplasm (Fig. 6A). Stable *PEX3-myc* transfectants revealed a proliferation of ER membranes in the majority of cells (Fig. 6B). In contrast to the untransfected cells, the ER layers were spread nearly throughout the cytoplasm. Furthermore, the morphology of the ER was different in that the ER network appeared to be more branched and in these branching points the cisternae seemed to be slightly dilated (Fig. 6B, bold arrows). These changes are consistent with the hypothesis that the targeting of Pex3p to the peroxisome is directed through the ER.

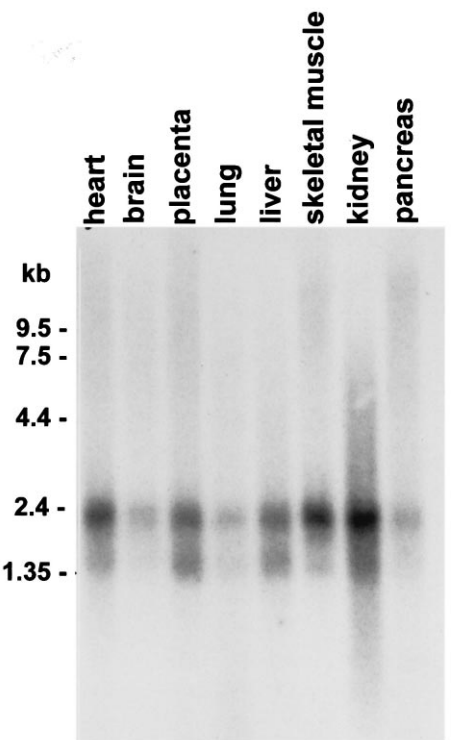


Fig. 3. Expression of the *PEX3* gene in various human tissues. A Northern blot of mRNA from several human tissues was hybridized with a cDNA fragment amplified from the coding sequence of the human *PEX3* gene. The probe detected signals at 1.6 and 2.3 kb. The lengths of the molecular weight marker fragments are indicated on the left.

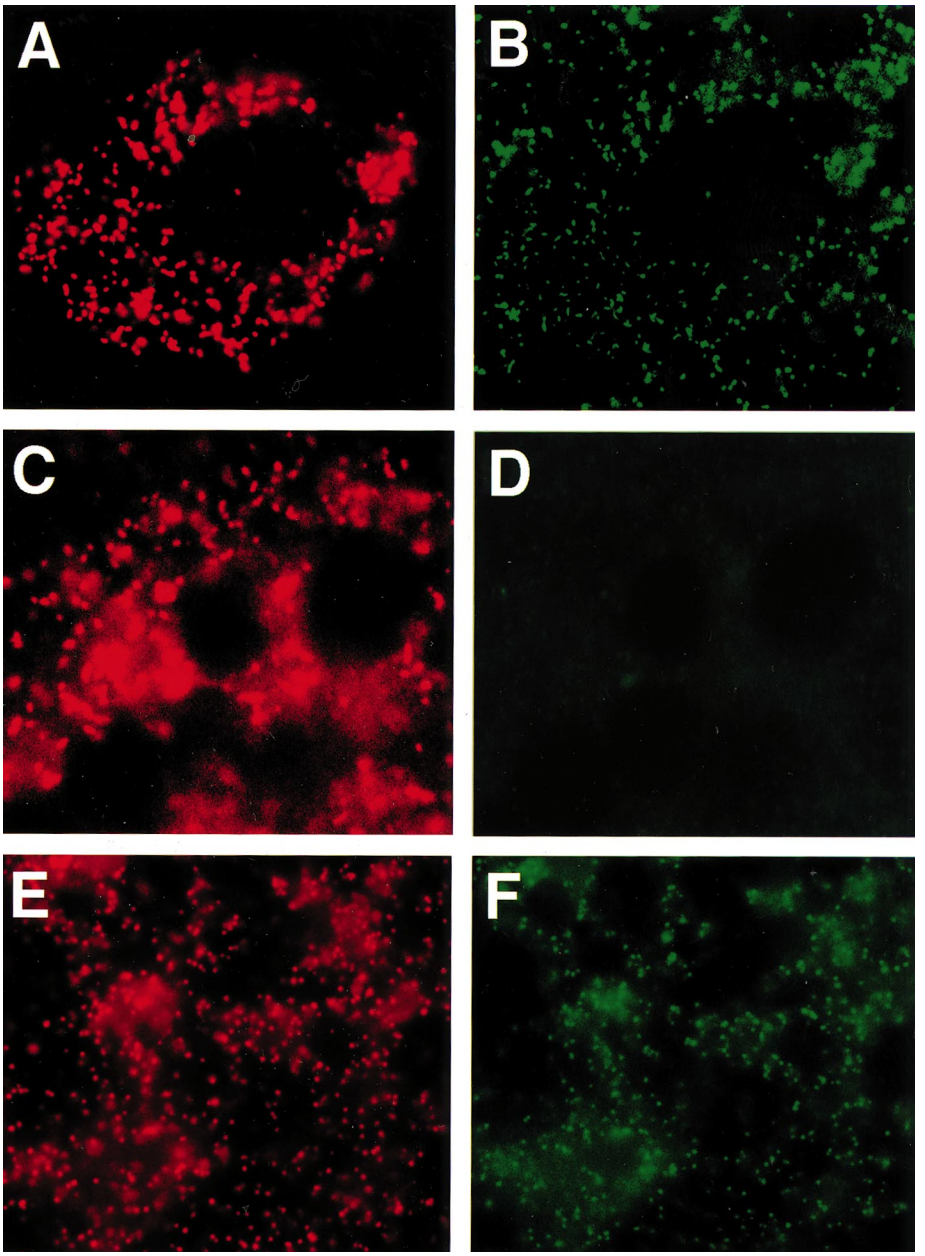


Fig. 4. Subcellular localization of the human Pex3p. Indirect immunofluorescence analysis of Pex3p-myc overexpressing COS7 cells. Permeabilization was performed with 1% Triton X-100 (A,B) or 25 μg/ml digitonin (C–F). Pretreated cells were co-stained with fluorescein conjugated IgG to anti-catalase antibodies (B,D) and rhodamine conjugated IgG to anti-myc antibodies (A,C) or with fluorescein conjugated IgG to anti-PMP70 antibodies (F) and rhodamine conjugated IgG to anti-myc antibodies (E). Note that the punctate structures stained with anti-myc antibodies co-localize with the peroxisomal marker enzyme catalase in Triton X-100 treated (A and B) and with PMP70 in digitonin treated cells (E and F). No catalase staining is seen in digitonin treated cells (C).

## 4. Discussion

We identified the human *PEX3* cDNA by homology probing using the amino acid sequence of the orthologous yeast protein published as Pas2p [19]. The 373 amino acid protein with a predicted molecular mass of 42.137 kDa shows a sequence similarity of 19.7–23.1% identical residues with the presumed orthologous peroxisomal assembly proteins from *S. cerevisiae*, *H. polymorpha* and *P. pastoris* (Fig. 1). As can be expected for a peroxisomal assembly protein, Pex3p is expressed in all human cells and tissues analyzed (Fig. 3). Transcripts derived from processing either near the poly(A) signal at position +1532 or at position +2179 correspond well to the signals at 1.6 and 2.3 kb detected by Northern blot analysis. Immunofluorescence analysis of transfected COS7 cells co-localized the human Pex3p with catalase (Fig. 4) and qualified it as a peroxisomal protein as was expected for an ortholog of the yeast Pex3 proteins.

The prediction of transmembrane segments for the human Pex3p is in general similar to that for the yeast proteins suggesting that the human Pex3p is also an integral membrane protein (Fig. 2). The HsPex3p amino acid sequence 109–131 shows a high prediction score indicative of a TM segment. Together with the second hydrophobic domain at amino acids

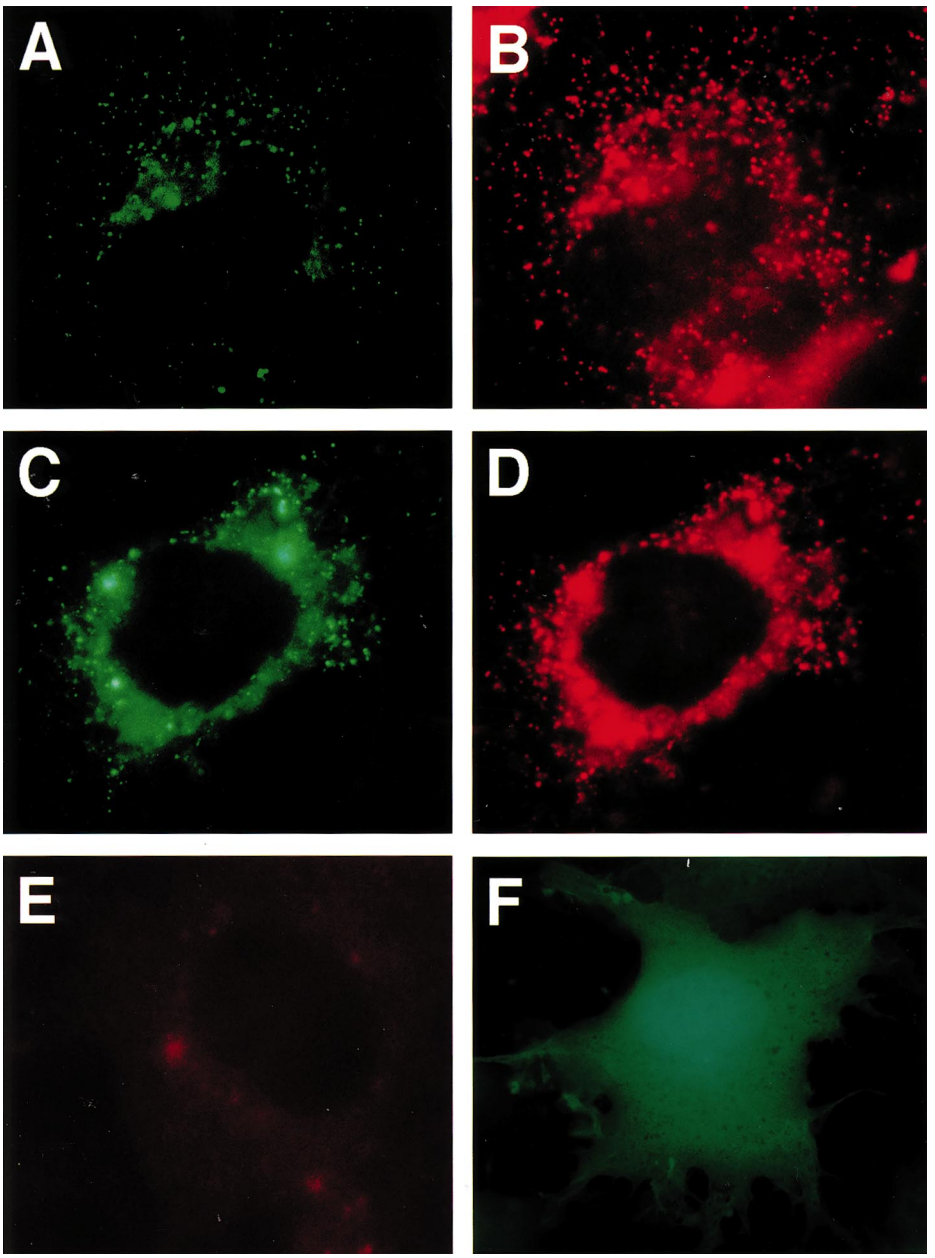


Fig. 5. Targeting analyses with the N-terminus of Pex3p. Indirect immunofluorescence analysis of Pex[1–40]GFP overexpressing COS7 cells. Permeabilization was performed with 1% Triton X-100 (A,B,F) or 25 μg/ml digitonin (C,D,E). The observed pattern was created by: A and C, Pex[1–40]GFP fluorescence; B and E, fluorescein conjugated IgG to anti-catalase antibodies; D, rhodamine conjugated IgG to anti-GFP antibodies; F, GFP fluorescence. Note that the punctate pattern produced by Pex[1–40]GFP co-localizes with the peroxisomal marker enzyme catalase in Triton X-100 pretreated cells (A and B). No punctate pattern was created by GFP without targeting signal (F).

15–33, the structure may conform to the model postulated for the yeast HpPex3p and ScPex3p [17,19] where the C-terminal portion of the protein is exposed to the cytosol while the N-terminus is fixed to the peroxisomal membrane by one membrane spanning and one anchoring segment. This hypothesis is confirmed by the topological data raised by immunofluorescence analyses using myc tagged (Fig. 4) and GFP tagged (Fig. 5) constructs. The detection of both reporter proteins on the cytoplasmic surface of the peroxisome suggests that the C-terminus (represented by the myc epitope) faces the cytosol while the N-terminus seems to be in the peroxisomal matrix. Since the amino acids 1–40 of Pex3p contain the first predicted TM segment and the GFP is fused to amino acid 40, the N-terminal amino acid might face the intraorganellar space.

Proteins destined for the peroxisomal matrix contain one of the defined targeting signals PTS1 and PTS2, which promote the import by interaction with the corresponding receptors [27], or a putative internal signal [28]. Targeting sequences of integral membrane proteins appear to be distinct but are less well defined. A putative targeting sequence for peroxisomal membrane proteins has been identified in a loop between two TM segments of PMP47 from *Candida boidinii* which includes the positively charged motif KIKKR [29,30]. We demonstrated that the N-terminal 40 amino acids of the human Pex3p are sufficient to target GFP to the peroxisome.

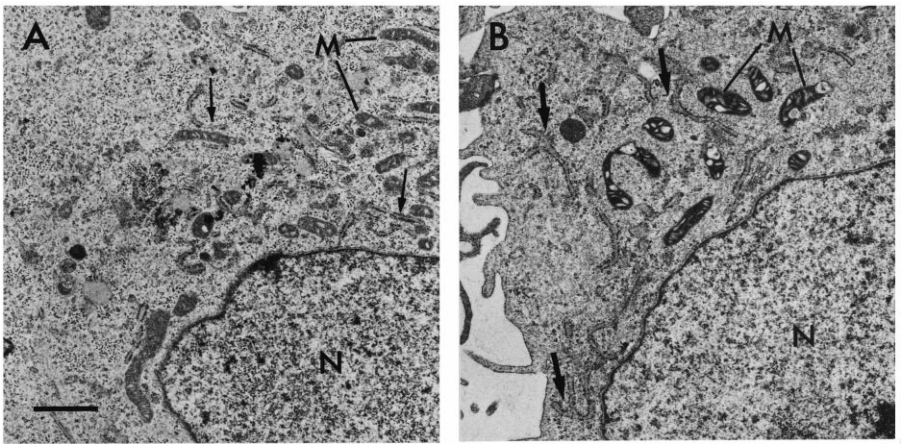


Fig. 6. Morphology of Pex3p overexpressing mammalian cells. Electron microscopic analysis of untransfected (A) and Pex3p-myc expressing (B) COS7 cells. The ER membranes in Pex3 overexpressing cells appear more branched (bold arrows, B) compared to untransfected cells (small arrows, A). N, nucleus; M, mitochondria. Bar = 1 μm. Magnification ×8500.

This is consistent with data on yeast PpPex3p, where the N-terminal 40 amino acids were also shown to be able to bring GFP to the peroxisome [19]. Therefore, the authors searched for elements homologous to the PMP47 motif and identified the sequence QIKRR at position 53–57 of HpPex3p and 59–63 of ScPex3p, which is also 100% conserved in PpPex3p (Fig. 1, indicated as a). Because this motif is excluded from the first 40 amino acids, another positively charged sequence at position 10–15 (RNKKK) was discussed to be important for membrane targeting. The alignment presented in Fig. 1 revealed that this sequence is highly conserved in the human Pex3p (RHKKK) and also throughout all yeast species even though the homology of ScPex3p (RHRGK) is lower. A comparison of a nine amino acid sequence including this motif shows the consensus sequence (L/I/V) X R X (K/R) X K X (L/I). The positively charged motif is located within the N-terminal 16 amino acids which have been demonstrated to direct yeast HpPex3p to probably ER derived membranous structures and, with lower efficacy, to the peroxisome [18]. This sequence may potentially play a crucial role in peroxisomal targeting via the ER and hence in the biogenesis and maintenance of peroxisomal membranes. In contrast, the amino acid sequence QIKRR (Fig. 1, indicated as b) is not particularly conserved in the human Pex3 protein. The morphological analysis of Pex3-myc overexpressing COS7 cells revealed a proliferation of the ER which is shown by structural changes and higher abundance of ER membranes (Fig. 6). These findings may further support the idea that new peroxisomes may form by budding from the ER.

In summary, the human Pex3p was revealed to have the same subcellular location and similar structural features as the yeast orthologous proteins. Pex3p and Pex19p have been shown to interact in yeast [16]. The lack of residual peroxisomal ghosts in yeast cells being defective in one of these peroxins suggests that these two proteins (probably together with other peroxins) may collaborate in the early stages of peroxisomal membrane assembly. These early steps of peroxisome formation may be directed via the ER [18,22] which is supported by the ER morphology of HsPex3p overexpressing mammalian cells. Since disruption of human peroxins is involved in the pathogenesis of human peroxisomal biogenesis disorders, it seems reasonable to suggest that *PEX3* is indeed another gene implicated in one of these disorders.

*Acknowledgements:* We thank Dr. Ennes Auerswald for the opportunity to use the sequencing facility and Heide Hinz as well as Sabine Herzmann for excellent technical assistance. We are grateful to Dr. Ania Muntau and Peter Mayerhofer for helpful assistance in performing transfection experiments. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (RO 727/1-1).

## References

[1] van den Bosch, H., Schutgens, R.B.H., Wanders, R.J.H. and Tager, J.M. (1992) Annu. Rev. Biochem. 61, 157–197.
[2] Distel, B., Erdmann, R., Gould, S.J., Blobel, G., Crane, D.I., Cregg, J.M., Dodt, 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., Valle, D., van der Klei, I., van Veldhoven, P.P. and Veenhuis, M. (1996) J. Cell Biol. 135, 1–3.
[3] Lazarow, P.B. (1995) J. Neuropathol. Exp. Neurol. 54, 720–725.
[4] Lazarow, P.B. and Moser, H.W. (1995) in: The Metabolic and Molecular Bases of Inherited Disease (Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D., Eds.), Vol. II, pp. 2287–2324, McGraw-Hill, New York.
[5] Slawecki, M.L., Dodt, G., Steinberg, S., Moser, A.B., Moser, H.W. and Gould, S.J. (1995) J. Cell Sci. 108, 1817–1829.
[6] Tateishi, K., Okumoto, K., Shimozawa, N., Tsukamoto, T., Osumi, T., Suzuki, Y., Kondo, N., Okano, I. and Fujiki, Y. (1997) Eur. J. Cell Biol. 73, 352–359.
[7] Dodt, G., Braverman, N., Wong, C., Moser, A., Moser, H.W., Watkins, P., Valle, D. and Gould, S. (1995) Nature Genet. 9, 115–125.
[8] Braverman, N., Steel, G., Obie, C., Moser, A., Moser, H., Gould, S.J. and Valle, D. (1997) Nature Genet. 15, 369–376.
[9] Motley, A.M., Hettema, E.H., Hogenhout, E.M., Brites, P., ten Asbroek, A.L.M.A., Wijburg, F.A., Baas, F., Heijmans, H.S., Tabak, H.F., Wanders, R.J.A. and Distel, B. (1997) Nature Genet. 15, 377–380.
[10] Purdue, P.E., Zhang, J.W., Skoneczny, M. and Lazarow, P.B. (1997) Nature Genet. 15, 381–384.
[11] Reuber, B.E., Germain-Lee, E., Collin, C.S., Morrell, J.C., Ameritunga, R., Moser, H.W., Valle, D. and Gould, S.J. (1997) Nature Genet. 17, 445–448.
[12] Portsteffen, H., Beyer, A., Becker, E., Epplen, C., Pawlak, A., Kunau, W.-H. and Dodt, G. (1997) Nature Genet. 17, 449–452.

[13] Yahraus, T., Braverman, N., Dodt, G., Kalish, J.E., Morell, J.C., Moser, H.W., Valle, D. and Gould, S.J. (1996) EMBO J. 15, 2914–2923.
[14] Shimozawa, N., Tsukamoto, T., Suzuki, Y., Orii, T., Shirayoshi, Y., Mori, T. and Fujiki, Y. (1992) Science 255, 1132–1134.
[15] Chang, C.-C., Lee, W.-H., Moser, H., Valle, D. and Gould, S.J. (1997) Nature Genet. 15, 385–388.
[16] Götte, K., Girzalsky, W., Linkert, M., Baumgart, E., Kammerer, S., Kunau, W.-H. and Erdmann, R. (1998) Mol. Cell. Biol. 18, 616–628.
[17] Höhfeld, J., Veenhuis, M. and Kunau, W.-H. (1991) J. Cell Biol. 114, 1167–1178.
[18] Baerends, R.J.S., Rasmussen, S.W., Hilbrands, R.E., van der Heide, M., Faber, K.N., Reuvekamp, P.T.W., Kiel, J.A.K.W., Cregg, J.M., van der Klei, I.J. and Veenhuis, M. (1996) J. Biol. Chem. 271, 8887–8894.
[19] Wiemer, E.A.C., Lüers, G.H., Faber, K.N., Wenzel, T., Veenhuis, M. and Subramani, S. (1996) J. Biol. Chem. 271, 18973–18980.
[20] Lazarow, P.B. and Fujiki, Y. (1985) Annu. Rev. Cell Biol. 1, 489–530.
[21] Waterham, H.R., Titorenko, V.I., Swaving, G.J., Harder, W. and Veenhuis, M. (1993) EMBO J. 12, 4785–4794.
[22] Elgersma, Y., Kwast, L., van den Berg, M., Snyder, W.B., Distel, B., Subramani, S. and Tabak, H.F. (1997) EMBO J. 16, 7326–7341.
[23] Braun, A., Kammerer, S., Weissenhorn, W., Weiss, E.H. and Cleve, H. (1994) Gene 146, 291–295.
[24] Kammerer, S., Arnold, N., Gutensohn, W., Mewes, H.-W., Kunau, W.-H., Höfler, G., Roscher, A.A. and Braun, A. (1997) Genomics 45, 200–210.
[25] Boguski, M., Lowe, T.M.J. and Tolstoshev, C.M. (1993) Nature Genet. 4, 332–333.
[26] Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Bonfield, J., Burton, J., Connell, M., Copsey, T., Cooper, J., Coulson, A., Craxton, M., Dear, S., Du, Z., Durbin, R., Favello, A., Fraser, A., Fulton, L., Gardner, A., Green, P., Hawkins, T., Hillier, L., Jier, M., Johnston, L., Jones, M., Kershaw, J., Kirsten, J., Laisster, N., Latreille, P., Lightning, J., Lloyd, C., Mortimore, B., O'Callaghan, M., Parson, J., Percy, C., Rifken, L., Roopra, A., Saunders, D., Shownkeen, R., Sims, M., Smaldon, N., Smith, A., Smith, M., Sonnhammer, E., Staden, R., Sulston, J., Thierry-Mieg, J., Thomas, K., Vaudin, M., Vaughan, K., Waterston, R., Watson, A., Weinstock, L., Wilkinson-Sproat, J. and Wohldman, P. (1994) Nature 368, 32–38.
[27] Subramani, S. (1997) Nature Genet. 15, 331–333.
[28] Small, G.M., Szabo, L.J. and Lazarow, P.B. (1988) EMBO J. 7, 1167–1173.
[29] McCammon, M.T., McNew, J.A., Willy, P.J. and Goodman, J.M. (1994) J. Cell Biol. 124, 915–925.
[30] Dyer, J.M., McNew, J.A. and Goodman, J.M. (1996) J. Cell Biol. 133, 269–280.
[31] Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) Nucleic Acids Res. 22, 4673–4680.
[32] Hofmann, K. and Stoffel, W. (1993) Biol. Chem. Hoppe-Seyler 347, 166.
[33] Church, G.M. and Gilbert, W. (1984) Proc. Natl. Acad. Sci. USA 81, 1991–1995.