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The Peroxin Pex17p of the Yeast *Yarrowia lipolytica* Is Associated Peripherally with the Peroxisomal Membrane and Is Required for the Import of a Subset of Matrix Proteins

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***PEX* genes encode peroxins, which are required for the biogenesis of peroxisomes. The *Yarrowia lipolytica PEX17* gene encodes the peroxin Pex17p, which is 671 amino acids in length and has a predicted molecular mass of 75,588 Da. Pex17p is peripherally associated with the peroxisomal membrane. The carboxyl-terminal tripeptide, Gly-Thr-Leu, of Pex17p is not necessary for its targeting to peroxisomes. Synthesis of Pex17p is low in cells grown in glucose-containing medium and increases after the cells are shifted to oleic acid-containing medium. Cells of the *pex17-1* mutant, the original mutant strain, and the *pex17-KA* mutant, a strain in which most of the *PEX17* gene is deleted, fail to form normal peroxisomes but instead contain numerous large, multimembraned structures. The import of peroxisomal matrix proteins in these mutants is selectively impaired. This selective import is not a function of the nature of the peroxisomal targeting signal. We suggest a regulatory role for Pex17p in the import of a subset of matrix proteins into peroxisomes.**

The eukaryotic cell has developed an intricate network of organelles, with each being responsible for a specific subset of functions. Peroxisomes belong to the microbody family of organelles. They are the site of a diverse set of metabolic pathways, which vary depending on the organism and environmental conditions. Functions that have been conserved in peroxisomes from yeasts to humans include the β-oxidation of fatty acids and the decomposition of H_2O_2 by catalase (reviewed in references 19 and 29).

Peroxisomes are crucial for normal human development and physiology, as emphasized by a group of genetic disorders collectively referred to as the peroxisome biogenesis disorders, which lead to death in early infancy (20). Peroxisome biogenesis disorders are characterized by the failure of cells to assemble functional peroxisomes. For this reason, much work has been directed towards the elucidation of the peroxisome assembly pathway. Peroxisomes have been shown to develop from preexisting peroxisomes or from a peroxisome reticulum (19), although de novo assembly of peroxisomes has also been proposed (37). As a peroxisome grows and develops, it must import proteins into its matrix. All peroxisomal proteins are encoded in the nucleus and, with one apparent exception (6), are synthesized on free polysomes and imported posttranslationally in an ATP-dependent manner (for reviews, see references 19 and 29). Three types of peroxisomal targeting signal (PTS) have been characterized for matrix proteins (reviewed in references 7 and 27 to 29). PTS1 is a tripeptide at the extreme carboxyl termini of proteins, having the consensus sequence (Ser/Ala/Cys)(Lys/Arg/His)(Leu/Met). PTS2 is a nonapeptide appearing at or near the amino termini of a limited number of peroxisomal proteins and has the consensus sequence (Arg/Lys)(Leu/Val/Ile)$(X)_5$(His/Gln)(Leu/Ala). Some matrix proteins are targeted by internal PTSs that remain largely uncharacterized. The one peroxisomal membrane protein PTS so far characterized consists of a stretch of positively charged amino acid residues located on a hydrophilic loop of the protein facing the matrix (9).

Many proteins required for the assembly of peroxisomes have been isolated by the complementation of mutants defective in peroxisome biogenesis (*pex* mutants) in different model systems. The proteins encoded by *PEX* genes have been termed peroxins (8). To date, 13 peroxins have been identified, three of which have been shown to be defective in human peroxisome biogenesis disorders (reviewed in reference 23). Various yeasts have proven to be excellent experimental systems in which to study peroxisome assembly, because the lack of functional peroxisomes is not lethal on rich media and because the proliferation of peroxisomes can be induced by changing the growth substrate to one requiring peroxisomes for its metabolism (reviewed in references 29 and 34). Our laboratory has characterized a number of *PEX* genes of the yeast *Yarrowia lipolytica*, including *PEX2* (11, 32), *PEX5* (30), *PEX6* (25), and *PEX9* (10). Here we describe the characterization of the *PEX17* gene of *Y. lipolytica* and its peroxin.

MATERIALS AND METHODS

Strains and culture conditions. The yeast strains used in this study are listed in Table 1. Strains were grown in complete (YEPD and YPBO) or supplemented minimal (YND and YNO) media, as required. Medium components were as follows: YEPD, 1% yeast extract, 2% peptone, and 2% glucose; YPBO, 0.3% yeast extract, 0.5% peptone, 0.5% K_2HPO_4, 0.5% KH_2PO_4, 1% Brij 35, and 1% (wt/vol) oleic acid; YND, 0.67% yeast nitrogen base without amino acids and 2% glucose; and YNO, 0.67% yeast nitrogen base without amino acids, 0.05% (wt/vol) Tween 40, and 0.1% (wt/vol) oleic acid. Liquid YNO and YND media were supplemented with Complete Supplement Mixture (minus the appropriate amino acids) (Bio 101, Vista, Calif.), as per the manufacturer's instructions. YNO and YND agar plates were supplemented with leucine, uracil, lysine, and histidine, each at 50 μg/ml, as required. Growth was at 30°C.

Isolation of the *pex17-1* mutant strain. Wild-type *Y. lipolytica* E122 cells were chemically mutagenized with 1-methyl-3-nitro-1-nitrosoguanidine, and the *pex17-1* strain was initially isolated by its inability to utilize oleic acid as the carbon source (26). Further screening of the *pex17-1* strain included analysis by electron microscopy (25, 35) and subcellular fractionation (1). Mutant strains were characterized by standard genetic techniques for *Y. lipolytica* (14).

Isolation and DNA sequencing of the *PEX17* gene. The *PEX17* gene was isolated from a library of *Y. lipolytica* genomic DNA in the autonomously replicating *Escherichia coli* shuttle vector pINA445 (26) by functional complemen-

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TABLE 1. *Y. lipolytica* strains used in this study

Strain[a]	Genotype
E122	*MATA ura3-302 leu2-270 lys8-11*
22301-3	*MATB ura3-302 leu2-270 his1*
pex17-1	*MATA ura3-302 leu2-270 lys8-11 pex17-1*
PEX17	*MATA ura3-302 leu2-270 lys8-11 pex17-1* pLD50(*LEU2*)
pex17-KA	*MATA ura3-302 leu2-270 lys8-11 pex17::URA3*
pex17-KB	*MATB ura3-302 leu2-270 his1 pex17::URA3*
PEX17-HA	*MATA ura3-302 leu2-270 lys8-11 PEX17-HA*
D-17OB	*MATA/MATB ura3-302/ura3-302 leu2-270/leu2-270 lys8-11/+ +/his1 pex17-1/+*
D-17O17K	*MATA/MATB ura3-302/ura3-302 leu2-270/leu2-270 lys8-11/+ +/his1 pex17-1/pex17::URA3*
D-A17K	*MATA/MATB ura3-302/ura3-302 leu2-270/leu2-270 lys8-11/+ +/his1 +/pex17::URA3*
D-17KB	*MATA/MATB ura3-302/ura3-302 leu2-270/leu2-270 lys8-11/+ +/his1 pex17::URA3/+*
D-17K17K	*MATA/MATB ura3-302/ura3-302 leu2-270/leu2-270 lys8-11/+ +/his1 pex17::URA3/pex17::URA3*
D-AB	*MATA/MATB ura3-302/ura3-302 leu2-270/leu2-270 lys8-11/+ +/his1*

[a] Strains E122 and 22301-3 were from C. Gaillardin, Thiverval-Grignon, France. All other strains were from this study.

tation of the *pex17-1* mutant strain. The *pex17-1* strain was transformed by electroporation of DNA, and colonies that demonstrated both leucine prototrophy and the reestablished ability to grow on oleic acid as the carbon source (YNO agar) were selected (26). Complementing plasmids were recovered by transformation into *E. coli*, and one plasmid, pLD50, was chosen for further study. Standard recombinant DNA techniques were performed as described previously (3).

Overlapping restriction endonuclease fragments of the *PEX17* gene were isolated from the complementing vector pLD50 and ligated into the vector pGEM5Zf(+) or pGEM7Zf(+) (Promega, Madison, Wis.). Both strands of the minimal complementing gene fragment were sequenced by the dideoxynucleotide method with Sequenase 1.0 (U.S. Biochemical, Cleveland, Ohio). The sequence of the encoded protein, Pex17p, was deduced from the gene nucleotide sequence and compared to protein sequences deposited in the GENEINFO(R) BLAST network server of the National Center for Biotechnology Information (Bethesda, Md.). Additional analyses of DNA and protein sequences were done with the PC/GENE data analysis program, release 16.0 (IntelliGenetics, Mountain View, Calif.). Protein homology was determined by using the structure-genetics comparison matrix with an open gap cost of 4 and a unit gap cost of 4.

Integrative disruption of the *PEX17* gene. pLD50 was digested with *Sal*I to excise a fragment of ~2.4 kbp encompassing ~1.1 kbp of the open reading frame and an ~1.3-kbp fragment of the 5′ upstream region of the *PEX17* gene (see Fig. 3A). A *Sal*I fragment of ~1.7 kbp containing the *URA3* gene of *Y. lipolytica* was ligated into this site. This plasmid was digested with *Hin*dIII to liberate an ~4.8-kbp fragment containing the *URA3* gene flanked at its 5′ and 3′ ends by ~1.2 and ~1.8 kbp of *Y. lipolytica* genomic DNA, respectively. This linear fragment was introduced into the *Y. lipolytica* wild-type strains E122 and 22301-3 by electroporation. Strains that converted to uracil prototrophy and that were unable to use oleic acid as the carbon source were further characterized by Southern blotting. Two disruption strains, the *pex17-KA* and *pex17-KB* mutants, with E122 and 22301-3 backgrounds, respectively (Table 1), were chosen for further analysis.

Epitope tagging of Pex17p. An *Xba*I/*Hin*dIII fragment containing the open reading frame of the *PEX17* gene flanked by ~0.8 and ~1.0 kbp of genomic DNA at its 5′ and 3′ ends, respectively, was ligated into the plasmid pGEM7Zf(+). A fragment with *Sal*I termini, encoding the peptide DEDPLAMYPYDVPDYA AMYPYDVPDYAAMGKGES, which contains two repeats of the influenza virus hemagglutinin (HA) epitope (underlined residues) (16), was ligated into the unique *Sal*I site in the *PEX17* gene between the codons for amino acids 365 (Val) and 366 (Asp). This construct was digested with *Xba*I and *Hin*dIII to release a fragment containing the modified *PEX17* gene. The fragment was used to stably transform the *pex17-1* mutant strain at the *PEX17* locus. Transformants able to use oleic acid as the carbon source were further characterized by Southern blotting and immunoblotting. One strain, the *PEX17-HA* strain, having the tagged *PEX17* gene at the correct locus and synthesizing HA-tagged Pex17p (Pex17p-HA), was chosen for further study.

Mutagenesis of the *PEX17* gene. Site-directed mutagenesis was used to make a *PEX17* gene encoding a Pex17p lacking its carboxyl-terminal tripeptide (Pex17p-ΔGTL). A fragment with *Xba*I termini and containing the open reading frame of the *PEX17* gene was ligated into the *Xba*I site of pGEM7Zf(+). Site-directed mutagenesis was performed with the Sculptor in vitro mutagenesis system (Amersham Life Sciences, Oakville, Ontario, Canada), as per the manufacturer's instructions. The primer 5′ GGGCTACTCCCACGGTTAGTGCAC TCGAGCATTC 3′ was used to delete the nucleotides encoding the carboxyl-terminal Gly-Thr-Leu of Pex17p. Correct mutagenesis was confirmed by sequencing. The mutated region was excised with *Xba*I and *Sph*I and exchanged with the respective fragment of the *PEX17* gene in the plasmid pLD50. This plasmid construct was used to transform the *pex17-KA* disruption strain.

Peroxisome isolation, subfractionation, and extraction. A postnuclear supernatant (PNS) isolated from oleic acid-grown cells was fractionated by centrifugation at 20,000 × *g* into a pellet (20KgP) enriched for peroxisomes and mitochondria and a supernatant (20KgS), as described previously (1), except that homogenization of spheroplasts was performed in 5 mM MES (morpholineethanesulfonic acid) (pH 5.5) containing 1 M sorbitol, 1 mM KCl, 0.5 mM EDTA, 0.1% (vol/vol) ethanol, 1 mM phenylmethylsulfonyl fluoride, 5 mM NaF, 1 μg of leupeptin/ml, 1 μg of pepstatin/ml, and 1 μg of aprotinin/ml. These three fractions were analyzed for the distribution of peroxisomal and mitochondrial enzymes. Peroxisomes were purified from the 20KgP by isopycnic centrifugation on a discontinuous sucrose gradient (24). The protein concentration and sucrose density were determined, and analyses of marker enzymes for peroxisomes, mitochondria, the endoplasmic reticulum (ER), and vacuoles were performed for each subcellular fraction.

Subfractionation and extraction of peroxisomes were performed with the sucrose gradient fraction containing the peak of peroxisomal enzyme activity. Peroxisomes (~30 μg of protein) were lysed by the addition of 10 volumes of ice-cold Ti8 buffer (10 mM Tris-HCl [pH 8.0], 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 μg of leupeptin/ml, 1 μg of pepstatin/ml, 1 μg of aprotinin/ml) (15) and separated into a membrane fraction (Ti8P) and a soluble fraction (Ti8S), as described previously (30). The Ti8P was resuspended in ice-cold Ti8 buffer to a final protein concentration of 0.5 mg/ml. Half of the resuspension was subjected to extraction with 0.1 M Na_2CO_3 (pH 11.5) to yield a supernatant (CO_3S) enriched for soluble proteins and a pellet (CO_3P) enriched for membrane proteins (13, 30). Proteins of fractions and subfractions were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and analyzed by immunoblotting. When necessary, proteins were precipitated by the addition of trichloroacetic acid to 10% followed by washing of the precipitate in ice-cold 80% (vol/vol) acetone.

Antibodies. To produce antibodies to Pex17p, a 1.8-kbp fragment of the *PEX17* gene, which contains both the open reading frame and 3′ untranslated region and codes for amino acids 369 to 671 of Pex17p, was excised with *Bam*HI and *Hin*dIII and inserted into the corresponding sites of the vector pMAL-c2 (New England Biolabs, Beverly, Mass.) in frame and downstream of the *malE* gene, encoding maltose binding protein. Antibodies to the fusion protein were raised in rabbit, as previously described (10).

The 12CA5 monoclonal antibody, which recognizes a 9-amino-acid epitope of the influenza virus HA protein, was purchased from the Berkeley Antibody Company (Richmond, Calif.). Rabbit anti-SKL antibodies have been described previously (2). Guinea pig antibodies to *Y. lipolytica* peroxisomal thiolase and Pex2p and rabbit antibodies to *Y. lipolytica* peroxisomal isocitrate lyase and to *Saccharomyces cerevisiae* peroxisomal acyl coenzyme A (acyl-CoA) oxidase were described previously (11). Rabbit antibodies to *S. cerevisiae* malate synthase were kindly provided by A. Hartig (Institute of Biochemistry and Molecular Cell Biology, Vienna, Austria). Rabbit antibodies to *Y. lipolytica* Kar2p were kindly provided by D. Ogrydziak (University of California, Davis).

Protease protection. Protease protection analysis was performed as described previously (30). Aliquots (13 μg of protein) of the sucrose gradient fraction containing peak peroxisomal activity were incubated with increasing amounts of trypsin in the presence or absence of 0.5% (wt/vol) Triton X-100. Reaction mixtures were incubated on ice for 40 min, and reactions were terminated by addition of trichloroacetic acid to 10%. Protein precipitates were washed in ice-cold 80% (vol/vol) acetone. Equivalent fractions of each digestion were subjected to SDS-polyacrylamide gel electrophoresis followed by immunoblotting.

Analytical procedures. Enzymatic activities of catalase (22), fumarase (33), and alkaline phosphatase (31) were determined by established methods. Whole-cell lysates were prepared as described previously (26). Southern (3) and North-

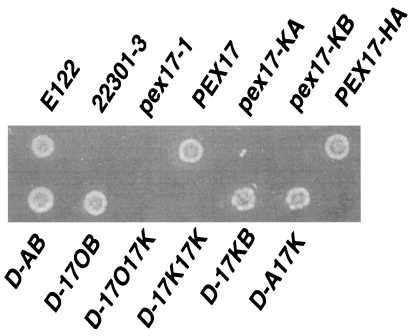


FIG. 1. Growth of various *Y. lipolytica* strains on oleic acid-containing medium. The strains used in this study (Table 1) were spotted onto YNO agar supplemented with leucine, uracil, and lysine, each at 50 μg/ml, and incubated for 2 days at 30°C. The appearance of the complemented *PEX17* strain is compared to those of the wild-type strains E122 and 22301-3, the original *pex17-1* mutant, the *pex17-KA* and *pex17-KB* gene disruption strains, the *PEX17-HA* strain synthesizing Pex17p-HA, and the *D-AB*, *D-17OB*, *D-17O17K*, *D-17K17K*, *D-17KB*, and *D-A17K* diploid strains. Strain 22301-3 was not supplemented for its auxotrophic requirements. Growth on YNO requires one functional copy of the *PEX17* gene.

ern (3, 30) blot analyses were performed according to established methods. SDS-polyacrylamide gel electrophoresis was performed as described previously (18). DNA and RNA were quantified by spectrophotometry. Protein concentrations were determined with a protein assay kit (Bio-Rad Laboratories, Mississauga, Ontario, Canada) with bovine serum albumin as a standard. Antigen-antibody complexes were detected by enhanced chemiluminescence (Amersham Life Sciences). Densitometry was performed with an Ultroscan XL laser densitometer (LKB Instruments, Bromma, Sweden). Quantification of densitometric signals was performed in the range in which the antigen concentration was linearly proportional to the densitometric signal.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been submitted to GenBank with accession no. U73028.

RESULTS

The *pex17-1* mutant strain lacks normal peroxisomes. The *pex17-1* mutant was initially identified by its inability to grow on medium containing oleic acid as the sole carbon source (Fig. 1). Biochemical characterization showed that the *pex17-1* mutant had deficiencies in the targeting and import of several peroxisomal matrix proteins (a detailed discussion follows), which conforms to the classical *pex* mutant phenotype.

The morphology of the *pex17-1* strain after incubation in medium containing oleic acid was compared to that of the wild-type E122 strain by electron microscopy (Fig. 2). The E122 strain showed several round peroxisomes surrounded by single unit membranes (Fig. 2A). Normal peroxisomes were absent in the *pex17-1* mutant strain. Instead, irregularly shaped structures surrounded by multiple unit membranes were observed (Fig. 2B).

Isolation and characterization of the *PEX17* gene. The *PEX17* gene was isolated by functional complementation of the *pex17-1* strain. The mutant strain was transformed with a plasmid library of *Y. lipolytica* genomic DNA, and 10 of approximately 10^5 transformants showed reestablished growth on a medium containing oleic acid as the sole carbon source. By restriction enzyme analysis of the complementing vectors, it was shown that three different vectors, each with a common overlapping fragment, were represented (data not shown). One vector, pLD50, which was present in the *PEX17* strain, was chosen for further study. The transformed *PEX17* strain showed reestablished growth on oleic acid (Fig. 1) and wild-type peroxisomal morphology (Fig. 2C). Complementation analysis was performed with fragments of pLD50 to narrow the complementing fragment from ~6.6 to ~3.5 kbp (Fig. 3A). Sequencing of this minimal complementing fragment revealed an open reading frame of 2,013 nucleotides, encoding a 671-amino-acid protein, Pex17p, with a predicted molecular mass of 75,588 Da (Fig. 3B).

Hydropathy analysis (17) showed Pex17p to be hydrophobic overall (data not shown). The sequence of Pex17p shows homology to those of peroxins encoded by the *PEX8* gene family. The Pex17p protein sequence is 19.9% identical and 37.4% similar to that *Hansenula polymorpha* Pex8p, a 71-kDa protein localized to the peroxisomal matrix and having both PTS1 and PTS2 motifs (36). Pex17p is 22.8% identical and 40.1% similar to *Pichia pastoris* Pex8p, an 81-kDa protein localized to the peroxisomal membrane and having a consensus PTS1 (21). Pex17p also shows 18.5% identity and 35.0% similarity to a yet-uncharacterized *S. cerevisiae* protein encoded by open reading frame YGR077c of chromosome VII (NCBI accession number Z72862).

The *PEX17* gene was disrupted in both the E122 and 22301-3 wild-type strains by integration of the *Y. lipolytica URA3* gene (Fig. 3A) to create the *pex17-KA* and *pex17-KB* strains, respectively (Table 1). These strains were unable to use oleic acid as the carbon source (Fig. 1) and had the same peroxisome morphology (Fig. 2D and E) and peroxisomal-protein-targeting defects (described below) as those of the original *pex17-1* mutant strain. The diploid *D-17OB*, *D-A17K*, and *D-17KB* strains (Table 1) were able to utilize oleic acid (Fig. 1), indicating the recessive nature of the *pex17-1* mutation and of the *PEX17* gene disruption. The *D-17O17K* diploid strain (Table 1), obtained from the crossing of the *pex17-KB* disruption strain with the *pex17-1* mutant strain, was unable to use oleic acid as a carbon source (Fig. 1), indicating that the authentic *PEX17* gene had been cloned.

Regulation of *PEX17* gene expression and Pex17p synthesis. For *PEX17* mRNA analysis, wild-type E122 cells were grown in glucose-containing YEPD medium for 14 h and then transferred to oleic acid-containing YPBO medium. Growth in YPBO medium was continued for 8 h. RNA was extracted from cells immediately prior to the shift to YPBO medium (0 h) and at 1-h intervals after the shift. Northern blot analysis was performed with equal amounts of RNA from each time point (Fig. 4A). mRNA encoding Pex17p was barely detectable in extracts of cells at 0 h but was readily visible at 1 h after cells were shifted to YPBO medium. Pex17p mRNA continued at elevated levels until the final time point at 8 h. The level of mRNA encoding Pex17p was qualitatively much lower than that of mRNA encoding thiolase, another peroxisomal matrix protein induced by growth in oleic acid (Fig. 4A). Pex17p mRNA levels in the *pex17-1* strain after the shift to YPBO medium were similar to those of the wild-type strain E122, showing that the mutation in the *pex17-1* strain did not affect transcription of the *PEX17* gene. As expected, no *PEX17* mRNA was detected in the *pex17-KA* disruption strain.

The synthesis of Pex17p was analyzed by using the *PEX17-HA* strain, which synthesizes an HA epitope-tagged version of Pex17p (Pex17p-HA). This strain was able to use oleic acid as the carbon source (Fig. 1) and exhibited normal peroxisome morphology (Fig. 2F). Immunoblotting with 12CA5 antibodies showed that Pex17p-HA was barely detectable in *PEX17-HA* cells grown in YEPD medium (Fig. 4B) and could be seen only after extended exposure of the immunoblot (data not shown). The level of Pex17p-HA increased after the cells were shifted to YPBO medium, peaked at 3 h after the shift, and thereafter decreased. Why the level of Pex17p-HA apparently decreases with time in YPBO medium, while the level of *PEX17* mRNA remains essentially constant, is unknown.

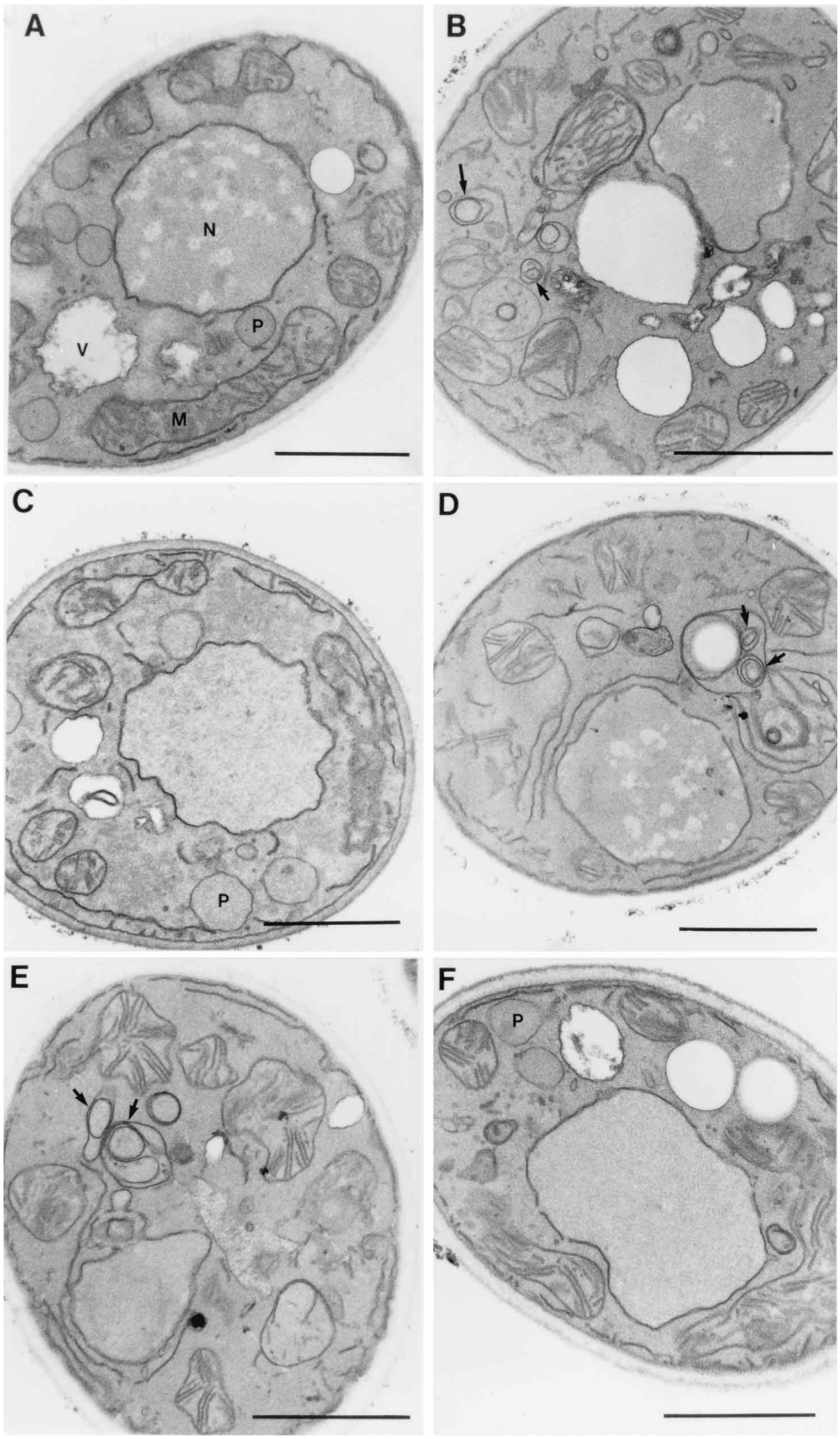
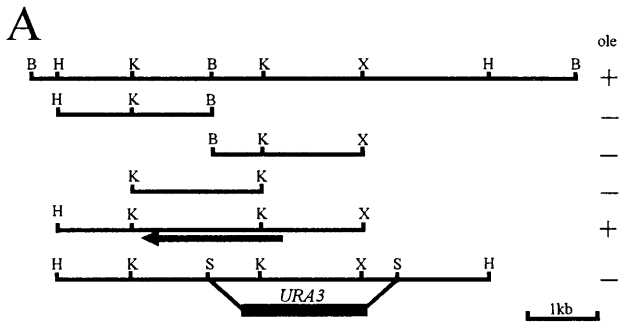


FIG. 2. Ultrastructure of the wild-type strain E122, the *pex17* mutants, and the *PEX17* complemented strains. The E122 (A), *pex17-1* (B), *PEX17* (C), *pex17-KA* (D and E), and *PEX17-HA* (F) strains were grown for 10 h in YEPD medium (YND for the *PEX17* strain), transferred to YPBO medium (YNO for the *PEX17* strain), and grown for an additional 8 h. Cells were fixed in 1.5% $KMnO_4$ and processed for electron microscopy. P, peroxisome; M, mitochondrion; N, nucleus; V, vacuole. Arrows, multimembraned structures. Bars, 1 μm.



B

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-146 TGAGTTCGATTCCCTCTCCGCCCACTTAAGGTATACTATGCACTCTAACATACCTCGGTTCCC
 -63 CGCATGTAACTTTCACGGACCTGCAACTCACAGAACTACCCCAACACCATGAACAAGTATCTA
                                                  M  N  K  Y  L    5
  16 GTGCCCCCGCCCCAGGCCAATAGAACGGTAACAAACCTCGATCTGCTCATCAACAACCTCCGT
     V  P  P  Q  A  N  R  T  V  T  N  L  D  L  L  I  N  N  L  R   26
  79 GGCTCCTCTACGCCCGGAGCCGCAGAGGTCGATACCAGGGATATCCTCCAACGAATCGTCTTT
     G  S  S  T  P  G  A  A  E  V  D  T  R  D  I  L  Q  R  I  V  F   47
 142 ATTCTCCCTACCATCAAGAACCCTCTCAACCTCGATCTGGTGATCAAGGAGATTATCAACTCG
     I  L  P  T  I  K  N  P  L  N  L  D  L  V  I  K  E  I  I  N  S   68
 205 CCACGCCCTTGGCCGCCTCTCATTGATCTCCATGACTACCAACAGCTCACAGACGCCTTCAGA
     P  R  P  W  P  P  L  I  D  L  H  D  Y  Q  Q  L  T  D  A  F  R   89
 268 GCAACCATCAAAAGAAAGGCCCTCGTCACAGACCCCACCATCAGCTTCGAGGCTTGGCTGGAG
     A  T  I  K  R  K  A  L  V  T  D  P  T  I  S  F  E  A  W  L  E  110
 331 ACCTGCTTCCAGGTCATTACTCGATTCGCAGGACCGGGTTGGAAGAAGCTACCTCTTCTGGCA
     T  C  F  Q  V  I  T  R  F  A  G  P  G  W  K  K  L  P  L  L  A  131
 394 GGATTGATTCTCGCAGATTACGACATTTCAGCTGATGGACCGACACTGGAACGAAAACCAGGT
     G  L  I  L  A  D  Y  D  I  S  A  D  G  P  T  L  E  R  K  P  G  152
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     F  P  S  K  L  K  H  L  L  K  R  E  F  V  T  T  F  D  Q  C  L  173
 520 TCTATCGACACCCGAAACCGAAGCGACGCCACAAAATGGGTACCTGTGCTTGCCTGCATTTCT
     S  I  D  T  R  N  R  S  D  A  T  K  W  V  P  V  L  A  C  I  S  194
 583 ATCGCACAGGTCTACTCGCTGCTAGGTGACGTTGCTATCAACTACAGACGGTTCTTGCAGGTG
     I  A  Q  V  Y  S  L  L  G  D  V  A  I  N  Y  R  R  F  L  Q  V  215
 646 GGTCTGGATCTCATCTTCTCAAACTACGGTTTGGAAATGGGCACGGCTCTGGCAAGACTCCAT
     G  L  D  L  I  F  S  N  Y  G  L  E  M  G  T  A  L  A  R  L  H  236
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     A  E  S  G  G  D  A  T  T  A  G  G  L  I  G  K  K  L  K  E  P  257
 772 GTTGTGGCTCTACTCAACACTTTTGCACACATCGCATCCTCCTGCATCGTTCACGTGGATATC
     V  V  A  L  L  N  T  F  A  H  I  A  S  S  C  I  V  H  V  D  I  278
 835 GACTACATTGACCGTATCCAGAACAAAATTATCCTAGTGTGTGAGAACCAGGCCGAGACCTGG
     D  Y  I  D  R  I  Q  N  K  I  I  L  V  C  E  N  Q  A  E  T  W  299
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     R  I  L  T  I  E  S  P  T  V  M  H  H  Q  E  S  V  Q  Y  L  K  320
 961 TGGGAGCTGTTCACCCTCTGTATCATTATGCAGGGAATTGCAAACATGCTGCTCACACAAAAG
     W  E  L  F  T  L  C  I  I  M  Q  G  I  A  N  M  L  L  T  Q  K  341
1024 ATGAACCAGTTCATGTACCTGCAGCTGGCATACAAACAGCTTCAGGCACTCCACAGCATCTAT
     M  N  Q  F  M  Y  L  Q  L  A  Y  K  Q  L  Q  A  L  H  S  I  Y  362
1087 TTCATTGTCGACCAAATGGGATCCCAGTTTGCTGCATATGACTACGTCTTCTTCTCAGCGATC
     F  I  V  D  Q  M  G  S  Q  F  A  A  Y  D  Y  V  F  F  S  A  I  383
1150 GATGTCCTGTTGTCGGAGTACGCTCCTTACATCAAGAACAGAGGCACCATCCCACCCAACAAG
     D  V  L  L  S  E  Y  A  P  Y  I  K  N  R  G  T  I  P  P  N  K  404
1213 GAGTTTGTGGCCGAAAGACTGGCCGCGAATCTCGCTGGAACTTCAAACGTGGGGAGTCATCTT
     E  F  V  A  E  R  L  A  A  N  L  A  G  T  S  N  V  G  S  H  L  425
1276 CCTATTGATCGCTCTCGAGTTCTGTTCGCTCTCAACTACTACGAACAGCTTGTCACCGTATGT
     P  I  D  R  S  R  V  L  F  A  L  N  Y  Y  E  Q  L  V  T  V  C  446
1339 CACGATAGCTGCGTAGAGACCATCATTTATCCCATGGCACGATCCTTCCTCTATCCCACAAGC
     H  D  S  C  V  E  T  I  I  Y  P  M  A  R  S  F  L  Y  P  T  S  467
1402 GACATTCAGCAACTTAAACCGCTGGTGGAAGCTGCTCACTCCGTGATTCTGGCTGGCCTGGCA
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1465 GTGCCTACCAACGCTGTTGTGAACGCTAAGCTCATCCCAGAGTACATGGGGGGAGTTCTTCCT
     V  P  T  N  A  V  V  N  A  K  L  I  P  E  Y  M  G  G  V  L  P  509
1528 TTGTTTCCGGGAGTGTTTTCATGGAACCAGTTCGTGTTGGCCATTCAGTCCATCGTTAACACT
     L  F  P  G  V  F  S  W  N  Q  F  V  L  A  I  Q  S  I  V  N  T  530
1591 GTCTCTCCTCCTTCTGAAGTGTTTAAAACCAACCAGAAGCTCTTCCGTCTTGTGTTGGATTCT
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1654 TTGATGAAAAAGTGCAGAGACACGCCCGTGGGCATTCCTGTGCCACACTCAGTCACAGTATCT
     L  M  K  K  C  R  D  T  P  V  G  I  P  V  P  H  S  V  T  V  S  572
1717 CAGGAACAGGAAGACATTCCTCCTACACAGCGAGCTGTGGTAATGCTGGCTCTCATCAACTCG
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1780 CTGCCCTACGTTGATATCCGGTCTTTTGAGTTGTGGCTGCAGGAGACGTGGAATATGATCGAG
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1843 GCCACTCCCATGCTTGCCGAAAACGCCCCCAACAAGGAGCTTGCGCATGCCGAGCACGAGTTT
     A  T  P  M  L  A  E  N  A  P  N  K  E  L  A  H  A  E  H  E  F  635
1906 CTGGTTCTGGAAATGTGGAAGATGATTTCCGGCAACATTGACCAGCGTCTCAATGATGTGGCC
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1969 ATTCGATGGTGGTACAAAAAGAATGCTCGAGTGCATGGAACGTTATAACCGTGGGAGTAGCCC
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     AAGTCCCCGAGGAGTCGGACATATCGGTAAAGACGTTAAATGCGAGACATCTCGCGTCTCTGG
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FIG. 3. Cloning and analysis of the *PEX17* gene. (A) Complementing activity, restriction map, and targeted gene disruption strategy for the *PEX17* gene. The top line represents the genomic insert in the original complementing vector, pLD50. The + and − symbols denote the ability (+) or inability (−) of an insert to confer growth on oleic acid (ole) to the *pex17-1* strain. The arrow shows the direction and position of the *PEX17* open reading frame. The site of integration of the *Y. lipolytica URA3* gene into the *PEX17* gene is shown in the bottom construct. B, *Bam*HI; H, *Hin*dIII; K, *Kpn*I; S, *Sal*I; X, *Xba*I. (B) Nucleotide sequence of the *PEX17* gene and deduced amino acid sequence of Pex17p.

Pex17p-HA is peripherally associated with the peroxisomal membrane. *PEX17-HA* cells were grown in oleic acid-containing medium for 8 h and subjected to subcellular fractionation. Peroxisomes were isolated by isopycnic centrifugation on a discontinuous sucrose gradient. Immunoblot analysis showed Pex17p-HA to be localized to peroxisomes and to be absent in the 20KgS fraction (Fig. 5A). Lysis of peroxisomes with Ti8 buffer followed by high-speed centrifugation showed Pex17p-HA to be localized exclusively to the pellet fraction enriched for membranes, as was the peroxisomal integral membrane protein Pex2p (Fig. 5B, lane Ti8P). This treatment liberated the matrix protein thiolase to the supernatant (Ti8S). Treat-

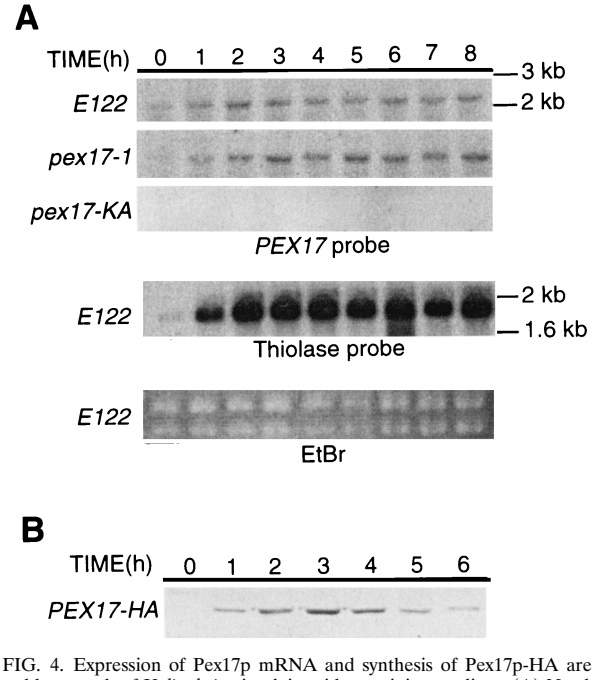

FIG. 4. Expression of Pex17p mRNA and synthesis of Pex17p-HA are induced by growth of *Y. lipolytica* in oleic acid-containing medium. (A) Northern blot analysis of Pex17p mRNA. Total RNA was isolated from the E122 wild-type strain and the *pex17-1* and *pex17-KA* mutant strains grown in YEPD medium (0 h) and at various time points after transfer to YPBO medium (1 to 8 h). Ten micrograms of RNA from each time point was separated on a formaldehyde agarose gel and transferred to nitrocellulose. Equal loading of RNA was ensured by ethidium bromide (EtBr) staining (data for the mutant strains not shown). The blots were hybridized with radiolabeled probes specific for the *PEX17* gene (*Sal*I-*Sph*I fragment, nucleotides 1095 to 1891 of the open reading frame) and the gene encoding peroxisomal thiolase (*Xho*I-*Bgl*II fragment, nucleotides 30 to 808 of the open reading frame) (5). The exposure times were 50 h for the Pex17p mRNA and 10 h for the thiolase mRNA. The numbers at right indicate the electrophoretic migrations of DNA markers. The numbers along the top indicate the time of oleic acid induction. (B) Immunoblot analysis of Pex17p-HA. Total cell lysates were made from the *PEX17-HA* strain grown in YEPD (0 h) and at various time points after transfer to YPBO. Forty micrograms of protein from each time point was subjected to electrophoresis on an SDS–10% polyacrylamide gel and transferred to nitrocellulose for immunoblot analysis with anti-HA monoclonal antibodies (12CA5). The numbers along the top indicate the time of oleic acid induction.

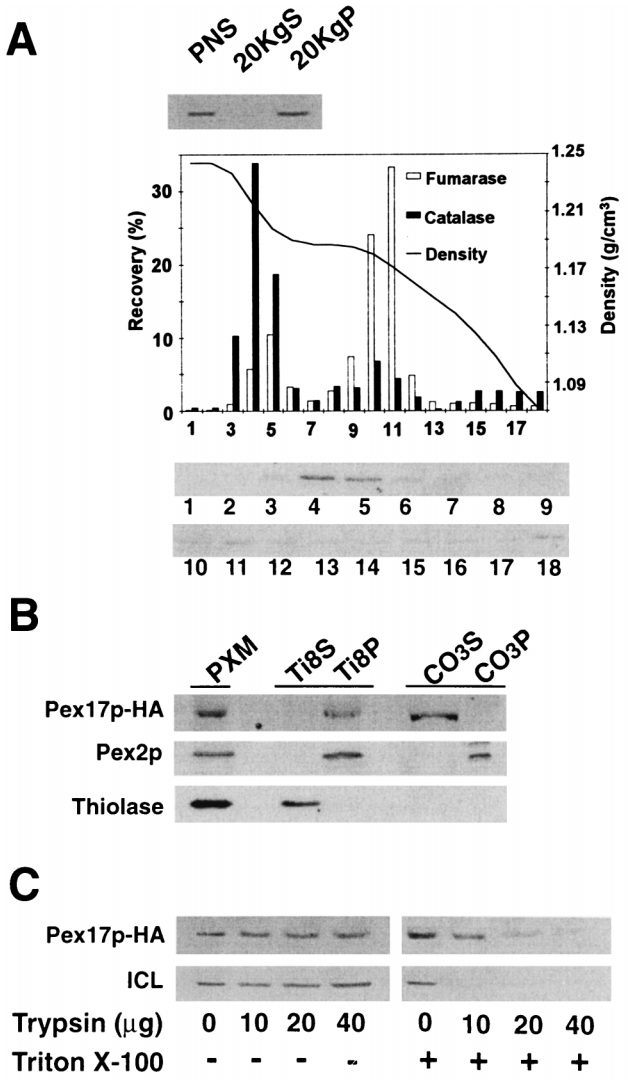

FIG. 5. Subcellular localization of Pex17p-HA. (A) Immunoblot analysis with anti-HA monoclonal antibodies (12CA5) of PNS, 20KgS, and 20KgP subcellular fractions (0.2% of the total volume of each) and of fractions of a sucrose density gradient (2% of the total volume of each). The distributions of the marker enzymes catalase (peroxisomes) and fumarase (mitochondria) in the gradient, as well as the sucrose densities of fractions, are also presented. Pex17p-HA is enriched in those fractions enriched for catalase. (B) Immunoblot analysis of whole peroxisomes (PXM) and peroxisomal subfractions (Ti8S, Ti8P, CO_3S, and CO_3P) of the *PEX17-HA* strain grown for 8 h in YPBO medium. Purified peroxisomes (30 μg of protein) were lysed with Ti8 buffer and subjected to centrifugation to yield a 245,000 × g_{max} supernatant (Ti8S) and pellet (Ti8P). CO_3S and CO_3P correspond to the 245,000 × g_{max} supernatant and pellet, respectively, recovered after treatment of Ti8P with 0.1 M Na_2CO_3 (pH 11.5). Blots were probed with antibodies to detect Pex17p-HA (12CA5), the peroxisomal integral membrane protein Pex2p, and the peroxisomal matrix protein thiolase. (C) Protease protection analysis. Whole peroxisomes (13 μg) isolated from the *PEX17-HA* strain were incubated with 1, 10, 20, or 40 μg of trypsin in the absence (−) or presence (+) of 0.5% (vol/vol) Triton X-100 for 40 min on ice. Samples were subjected to immunoblot analysis with anti-HA monoclonal antibodies (12CA5) to detect Pex17p-HA and with antibodies to the peroxisomal matrix protein isocitrate lyase (ICL).

ment of the Ti8P with 0.1 M Na_2CO_3 (pH 11.5), followed by high-speed centrifugation, liberated Pex17p-HA but not Pex2p to the supernatant fraction (Fig. 5B, compare lanes CO_3S and CO_3P), consistent with Pex17p-HA being associated with, but not integral to, the peroxisomal membrane.

A protease protection assay was performed with isolated peroxisomes to determine whether Pex17p-HA was preferentially associated with the matrix or cytosolic face of the peroxisomal membrane. Aliquots of peroxisomes were treated with increasing amounts of trypsin in the presence or absence of the nonionic detergent Triton X-100. Immunoblot analysis showed degradation of Pex17p-HA by trypsin in the presence, but not in the absence, of detergent, in a manner similar to that of the matrix protein isocitrate lyase (Fig. 5C). The entire Pex17p-HA was protected from digestion by the peroxisomal membrane. Our data suggest that Pex17p-HA is associated with the matrix face of the membrane; however, at this time we cannot entirely rule out the possibility that Pex17p-HA is associated with the cytosolic surface of the peroxisomal membrane and that the addition of detergent exposes hitherto inaccessible trypsin sites.

The carboxyl-terminal tripeptide Gly-Thr-Leu of Pex17p is not necessary for its targeting to peroxisomes. Pex17p does not contain consensus sequences for either PTS1 or PTS2. However, because of the extensive degeneracy of the PTS1 motif, we investigated the necessity of the carboxyl-terminal tripeptide Gly-Thr-Leu of Pex17p for its targeting to peroxisomes. The *pex17-KA* disruption strain was transformed with plasmids expressing either the wild-type *PEX17* gene or a mutated version of the gene encoding Pex17p with its carboxyl-terminal Gly-Thr-Leu deleted (Pex17p-ΔGTL). Pex17p-ΔGTL has at its carboxyl terminus the tripeptide Arg-Val-His (Fig. 3), which is dissimilar to the consensus PTS1. Expression of genes encoding either Pex17p or Pex17p-ΔGTL could functionally complement the *pex17-KA* strain, reestablishing growth on oleic acid-containing medium (Fig. 6). Therefore, the carboxyl-terminal tripeptide of Pex17p is not required for its activity. Subcellular fractionation demonstrated that both Pex17p and Pex17p-ΔGTL were exclusively localized to the 20KgP fraction enriched for peroxisomes (Fig. 6). Therefore, the carboxyl-terminal tripeptide of Pex17p is not necessary for its targeting to peroxisomes.

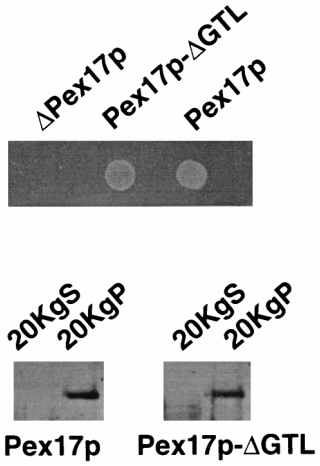

FIG. 6. The carboxyl-terminal tripeptide of Pex17p is not necessary for its activity or its targeting to peroxisomes. The appearance of the *pex17-KA* disruption strain (ΔPex17p) is compared to those of the *pex17-KA* strain transformed with plasmids expressing the genes encoding a Pex17p lacking its carboxyl-terminal tripeptide (Pex17p-ΔGTL) or full-length Pex17p. Growth was for 2 days at 30°C on YNO agar (top panel). Equal cellular fractions of the 20KgS and 20KgP isolated from the *pex17-KA* strains transformed with plasmids expressing the genes encoding full-length Pex17p or Pex17p-ΔGTL were analyzed by immunoblotting with anti-Pex17p antibodies (bottom panels). Anti-Pex17p antibodies recognize a polypeptide of ~73 kDa on immunoblots of oleic acid-grown E122 cells but not *pex17-KA* cells (data not shown).

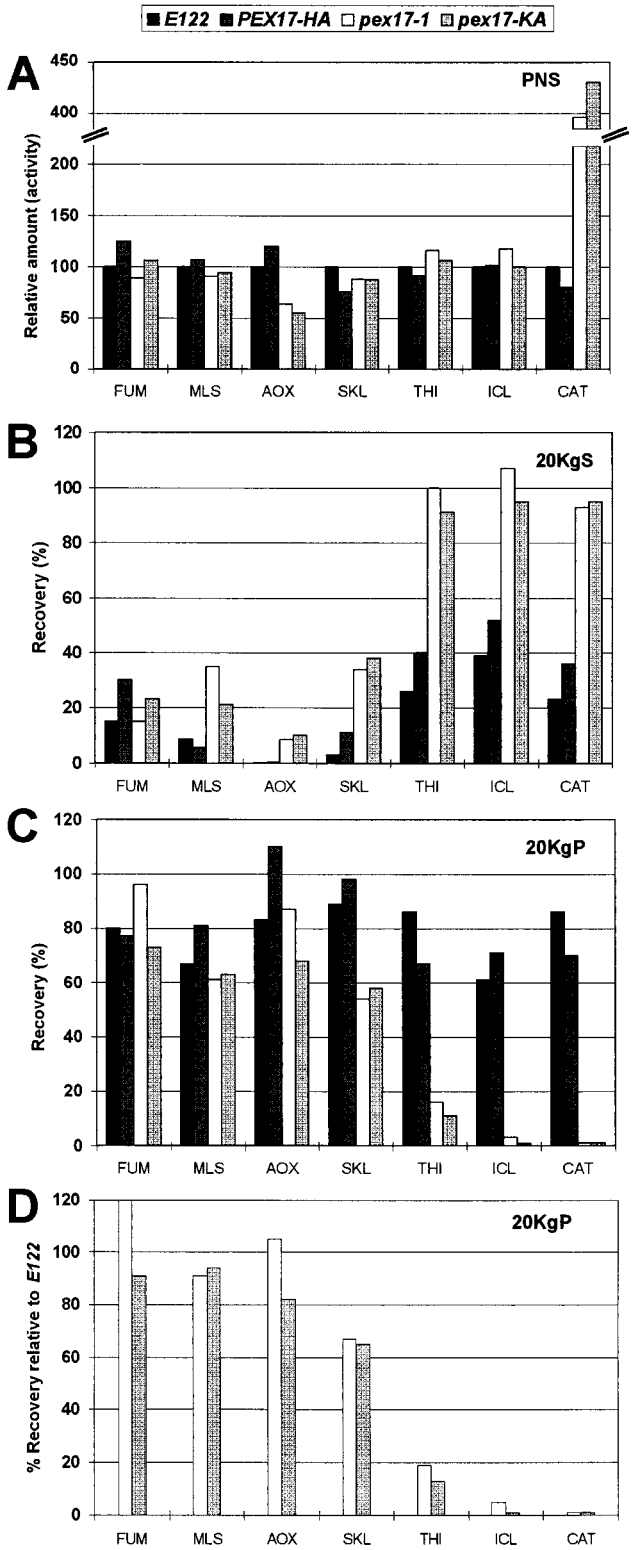

FIG. 7. Peroxisomal matrix proteins are mislocalized to the 20KgS to various extents in *pex17* mutants. Wild-type E122, *PEX17-HA*, *pex17-1*, and *pex17-KA* strains were grown in YPBO medium and subjected to subcellular fractionation, as described in Materials and Methods. Malate synthase (MLS), acyl-CoA oxidase (AOX), a 62-kDa anti-SKL-reactive polypeptide (SKL), thiolase (THI), and isocitrate lyase (ICL) were detected by immunoblot analysis with their respective antibodies. Immunoblots were quantitated by densitometry (arbitrary units) in the range at which the antigen concentration was linearly proportional to the densitometric signal. The distributions of fumarase (FUM) and catalase (CAT) were determined from their enzymatic activities. (A) Total amount (activity) of proteins in the PNSs of the four strains expressed relative to the total amount (activity) of proteins in the wild-type strain E122, which is taken as 100%. (B) Recovery of proteins in the 20KgS. (C) Recovery of proteins in the 20KgP. For panels B and C, recovery is reported as a percentage of the densitometric signal or enzymatic activity found in the PNS. Equal fractions of the 20KgS and 20KgP were analyzed for the distribution of matrix proteins. (D) Recovery of proteins in the 20KgPs of the *pex17-1* and *pex17-KA* mutant strains relative to their recovery in the 20KgP of the wild-type E122 strain, which is taken as 100%.

Mutations in the *PEX17* gene affect the localization of a subset of matrix proteins. To investigate the effects of mutation of the *PEX17* gene on peroxisomal matrix protein targeting, subcellular fractionation was performed with the E122, *PEX17-HA*, *pex17-1*, and *pex-17KA* strains grown for 9 h on oleic acid-containing medium. Under these conditions, the levels of peroxisomal matrix proteins analyzed (i.e., malate synthase, acyl-CoA oxidase, a 62-kDa anti-SKL-reactive polypeptide, thiolase, isocitrate lyase, and catalase) were greatly increased and reached steady state in the wild-type strain E122 (data not shown). The steady-state levels of most of these proteins in the *pex17-1* and *pex17-KA* strains were unchanged from the levels in the wild-type strain, except for acyl-CoA oxidase, which was at levels 55 to 65% of that in the wild-type strain, and catalase, which was at levels approximately four times that found in the wild-type strain (Fig. 7A). The reason for the highly elevated levels of catalase in the mutant strains is unknown.

The distributions of peroxisomal and mitochondrial proteins between the 20KgS and the 20KgP for the various strains are illustrated in Fig. 7B and C, respectively. In both the wild-type strain E122 and the *PEX17-HA* strain, peroxisomal and mitochondrial proteins were associated primarily with the 20KgP. In the *pex17-1* and *pex17-KA* mutant strains, the mitochondrial marker enzyme fumarase was localized primarily to the 20KgP, as in the E122 and *PEX17-HA* strains. However, the distributions of peroxisomal matrix proteins in the *pex17-1* and *pex17-KA* strains were different from those in E122 (Fig. 7D). While the mutant strains showed little impairment in their ability to import malate synthase and acyl-CoA oxidase (both were imported at greater than 80% of the efficiency in wild-type cells), the import of other matrix proteins was affected to various greater degrees. A 62-kDa anti-SKL-reactive protein (30) was mislocalized to the 20KgS to an intermediate extent (61 to 65% of the level in the 20KgP from the wild-type strain). Thiolase was more severely mislocalized to the 20KgS (17 to 20% of the level in the 20KgP from the wild-type strain), while isocitrate lyase and catalase were almost exclusively mislocalized to the 20KgS (1 to 5% and 1 to 2% of the levels in the 20KgP from the wild-type strain for isocitrate lyase and catalase, respectively).

Peroxisomal proteins pelletable to the 20KgP of *pex17* mutant strains colocalize with marker proteins of mitochondria, the ER, and the yeast vacuole. 20KgPs prepared from oleic acid-grown E122, *pex17-1*, and *pex17-KA* strains were fractionated by isopycnic centrifugation on a discontinuous sucrose gradient. In the E122 strain, the peroxisomal markers catalase (Fig. 8) and the 62-kDa and 64-kDa anti-SKL-reactive polypeptides, thiolase, isocitrate lyase, and malate synthase (data not shown) colocalized and peaked in fraction 4 (density = 1.21 g/cm^3). The mitochondrial marker fumarase peaked in fraction 10 (density = 1.18 g/cm^3) (Fig. 8), while the ER marker Kar2p peaked in fraction 11 (density = 1.17 g/cm^3) (Fig. 8). Some trailing of Kar2p to both heavier and lighter fractions was evident. The vacuolar marker alkaline phospha-

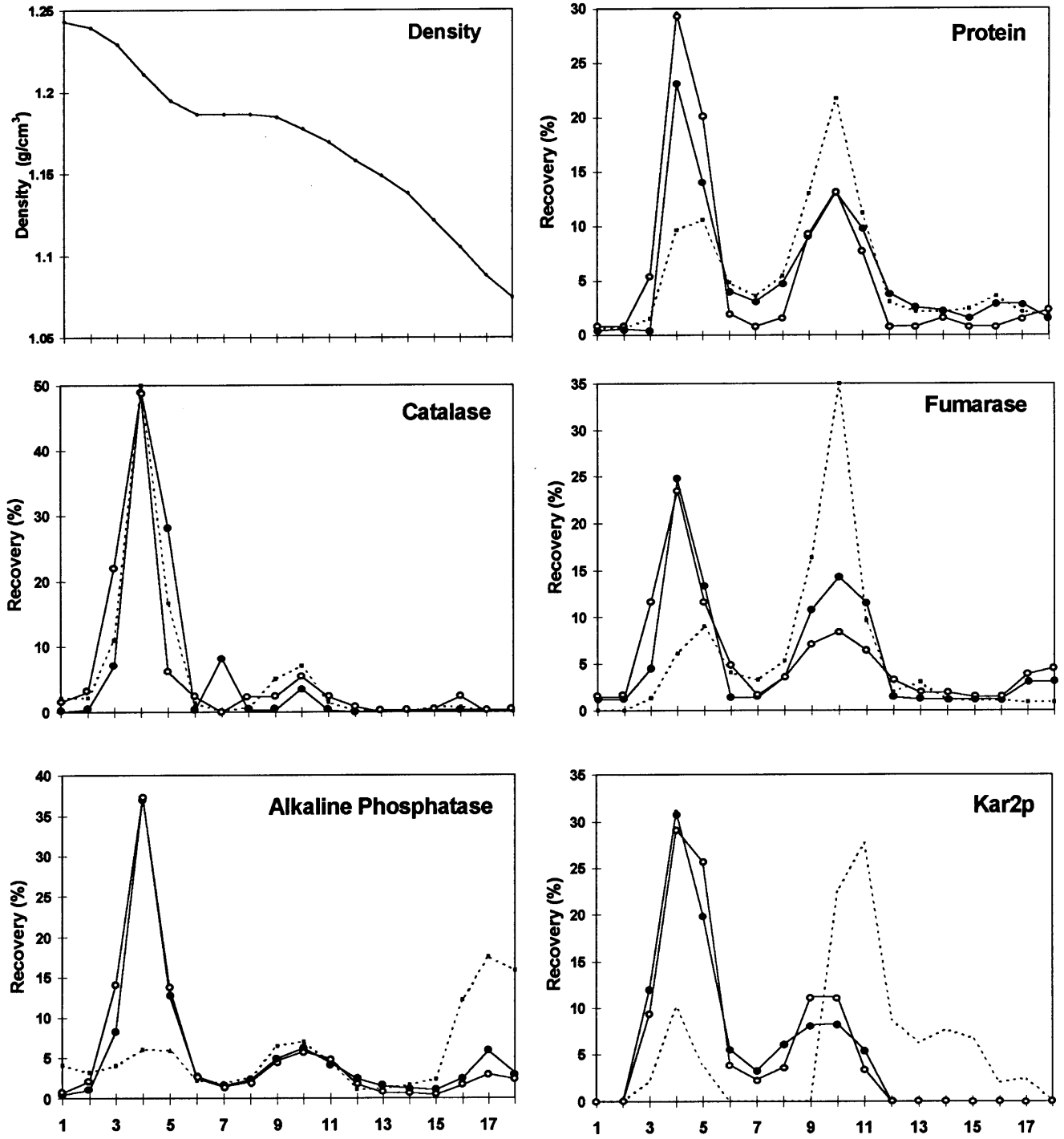

FIG. 8. Sucrose density gradient analysis of 20KgPs from the wild-type and *pex17* mutant strains. 20KgPs (3 mg of protein each) isolated from the E122, *pex17-1*, and *pex17-KA* strains were subjected to sucrose density gradient centrifugation, as described in Materials and Methods, and fractions were collected. Sucrose density and percent recoveries of loaded protein and of enzymatic activities in gradient fractions for the E122 (– – –), *pex17-1* (○), and *pex17-KA* (●) strains are diagrammed. The distributions of catalase, fumarase, and alkaline phosphatase were determined by measurement of their enzymatic activities. The distribution of Kar2p was determined by immunoblot analysis. Protein from equal volumes (1%) of each fraction was subjected to SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and probed with anti-Kar2p antibodies. Immunoblots were quantitated by densitometry, as described in the legend to Fig. 4.

tase showed a peak of activity in fraction 17 (density = 1.09 g/cm^3), with secondary peaks in fractions 4 and 10.

Analysis of the *pex17-1* and *pex17-KA* strains demonstrated a different distribution of marker proteins than seen for the wild-type strain (Fig. 8). All peroxisomal proteins tested colocalized and peaked in fraction 4, as for the wild-type strain (Fig. 8 shows data for catalase; data for other matrix enzymes not shown). Even the small amounts of catalase (Fig. 8) and

isocitrate lyase (data not shown) found in the 20KgP of the *pex17-1* and *pex17-KA* strains (Fig. 7) peaked in fraction 4. Fumarase and Kar2p also peaked in fraction 4. The presence of a large peak of alkaline phosphatase in fraction 4 from the *pex17-1* and *pex17-KA* mutant strains suggests that mutation of the *PEX17* gene may lead to increased autophagy. Colocalization of marker proteins of peroxisomes, mitochondria, and the ER in fraction 4 could result from the presence of elements of each of these organelles in autophagosomes, which may correspond to the multimembraned structures seen in electron micrographs of *pex17* cells (Fig. 2).

DISCUSSION

Here we report the isolation of *pex17* mutant strains of *Y. lipolytica*, their biochemical and morphological characterization, the cloning and sequencing of the *PEX17* gene, and the identification and characterization of the *PEX17* gene product, Pex17p.

Unlike the wild-type strain E122, *pex17* strains are unable to use oleic acid as the sole carbon source. Growth of *pex17* strains in oleic acid-containing media leads to the accumulation of structures surrounded by multiple unit membranes rather than to the formation of normal peroxisomes, which proliferate under these conditions in the wild-type strain. The membrane proliferation seen in the *pex17* mutants is common to other *pex* mutant strains of *Y. lipolytica*, including *pex5* (30) and *pex2* (11) mutants. Our morphological results demonstrate that *pex17* mutant strains are defective in peroxisome assembly.

Our biochemical results confirm that *pex17* strains are peroxisome assembly mutants. *pex17* mutants are impaired in their ability to correctly import a subset of peroxisomal matrix proteins. Results of subcellular fractionation show that the import of most matrix proteins by *pex17* mutants is impaired to variable extents compared to their import in wild-type cells. Catalase and isocitrate lyase are almost completely mislocalized, while thiolase and a 62-kDa anti-SKL-reactive polypeptide are only partially mislocalized. The import of malate synthase and acyl-CoA oxidase by *pex17* strains is comparable to that by the wild-type strain. This selective mislocalization is not due solely to the type of PTS in a particular matrix protein. For example, while the mislocalizations of a 62-kDa anti-SKL (PTS1)-reactive polypeptide and thiolase, a PTS2-containing protein (5), are only partial, isocitrate lyase, which contains a PTS1 (4), is completely mislocalized. The basis of this selective mislocalization is currently being investigated.

Interestingly, mutation of the *PEX17* gene results in the colocalization of marker proteins of the peroxisome, mitochondrion, ER, and vacuole, as determined by subcellular fractionation and density gradient centrifugation analysis. This colocalization could result from increased autophagy in *pex17* mutant strains, with elements of peroxisomes, mitochondria, and the ER undergoing digestion together in autophagosomes, which may correspond to the multimembraned structures seen in electron micrographs of *pex17* cells.

We have characterized the *PEX17* gene and its product, Pex17p. Pex17p is a 671-amino-acid protein with a predicted molecular mass of 75,588 Da. It has an overall hydrophobic character. Sodium carbonate extraction and protease protection analyses of fractions from the *PEX17-HA* strain suggest that Pex17p is peripherally associated with the matrix face of the peroxisomal membrane.

Database searches revealed some sequence identity between Pex17p and proteins of the Pex8p family of peroxins (21, 36). Proteins of this family are defined as 71- to 81-kDa peroxisome-associated proteins containing PTS1 motifs (8). Pex17p is probably not an immediate member of this family, for a number of reasons. The sequence identity of Pex17p with these peroxins is low (~20% identity with each member) compared to the identity between the two Pex8p members themselves (~40% identity). Moreover, the carboxyl-terminal tripeptide of Pex17p, unlike those of the members of the Pex8p family, does not fit the consensus PTS1 sequence and is not necessary for targeting to peroxisomes. In our experiments, Pex17p-ΔGTL could not target to peroxisomes by piggybacking on full-length Pex17p, as expression was done in the knockout *pex17-KA* strain. Although Pex8p of *H. polymorpha* (36) has functional PTS1 and PTS2 motifs, Pex17p does not have a consensus PTS2 sequence and is unlikely to be targeted to peroxisomes via that pathway. The homology between Pex17p and the Pex8p peroxins may result from a common functional domain(s) or a common structural feature(s) shared by this group of proteins.

In conclusion, *pex17* mutants mislocalize a subset of matrix proteins, suggesting that Pex17p functions in the import of these proteins into the peroxisome. Whether Pex17p plays a direct or indirect role in the import process remains to be determined. We are continuing to elucidate the role of Pex17p in peroxisomal protein import and peroxisome assembly by investigating both its spatial and temporal relationships with other peroxins that have been characterized previously.

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