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Pex11-related Proteins in Peroxisome Dynamics: A Role for the Novel Peroxin Pex27p in Controlling Peroxisome Size and Number in *Saccharomyces cerevisiae*

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Transcriptome profiling identified the gene *PEX25* encoding Pex25p, a peroxisomal membrane peroxin required for the regulation of peroxisome size and maintenance in *Saccharomyces cerevisiae*. Pex25p is related to a protein of unknown function encoded by the open reading frame, *YOR193w*, of the *S. cerevisiae* genome. Yor193p is a peripheral peroxisomal membrane protein that exhibits high sequence similarity not only to Pex25p but also to the peroxisomal membrane peroxin Pex11p. Unlike Pex25p and Pex11p, Yor193p is constitutively expressed in wild-type cells grown in oleic acid-containing medium, the metabolism of which requires intact peroxisomes. Cells deleted for the *YOR193w* gene show a few enlarged peroxisomes. Peroxisomes are greatly enlarged in cells harboring double deletions of the *YOR193w* and *PEX25* genes, the *YOR193w* and *PEX11* genes, and the *PEX25* and *PEX11* genes. Yeast two-hybrid analyses showed that Yor193p interacts with Pex25p and itself, Pex25p interacts with Yor193p and itself, and Pex11p interacts only with itself. Overexpression of *YOR193w*, *PEX25*, or *PEX11* led to peroxisome proliferation and the formation of small peroxisomes. Our data suggest a role for Yor193p, renamed Pex27p, in controlling peroxisome size and number in *S. cerevisiae*.

INTRODUCTION

Peroxisomes compartmentalize a diverse set of metabolic pathways involved in the inactivation of toxic substances, the regulation of cellular oxygen, and the metabolism of lipids, nitrogen bases, and carbohydrates. Peroxisomes are essential for normal human development and physiology. This fact is underscored by the lethality of a group of genetic disorders collectively called the peroxisome biogenesis disorders (PBDs) in which peroxisomes fail to assemble correctly. Defining the molecular bases of the PBDs has been the impetus behind the identification of the genes controlling peroxisome assembly, the *PEX* genes. To date, 25 *PEX* genes have been identified, and mutations in 11 of the 13 human orthologs have been shown to give rise to the PBDs (for reviews, see Lazarow and Fujiki, 1985; van den Bosch *et al*., 1992; Fujiki, 2000; Gould and Valle, 2000; Subramani *et al*., 2000; Purdue and Lazarow, 2001; Titorenko and Rachubinski, 2001; Brosius and Gärtner, 2002).

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Abbreviations used: 20KgP, 20,000 × *g* pellet; 20KgS, 20,000 × *g* supernatant; DsRed, red fluorescent protein from *Discosoma* sp.; ORF, open reading frame; PBD, peroxisome biogenesis disorder; PNS, postnuclear supernatant; PTS, peroxisome targeting signal.

Peroxisomes themselves do not carry any genetic material and do not synthesize proteins. All peroxisomal proteins are encoded by nuclear genes and synthesized on cytosolic polysomes. Most soluble proteins of the peroxisomal matrix are targeted to the peroxisome by a peroxisome targeting signal 1 (PTS1), a tripeptide located at the extreme carboxyl termini of proteins. A much smaller subset of proteins is targeted by a PTS2, a nonapeptide located near or at the amino termini of proteins. A few peroxisomal matrix proteins are targeted by largely uncharacterized internal PTSs. Pex5p and Pex7p are the receptors for PTS1 and PTS2, respectively, and various proteins, including Pex13p and Pex14p, form a docking complex at the peroxisomal membrane for these receptors. The sorting of peroxisomal membrane proteins is much less well understood and seems independent of that of matrix proteins (for reviews, see Subramani, 1998; Hettema *et al*., 1999; Subramani *et al*., 2000; Terlecky and Fransen, 2000; Purdue and Lazarow, 2001; Titorenko and Rachubinski, 2001).

In contrast to protein sorting to peroxisomes, much less is known about the mechanism of peroxisome proliferation and the molecular players involved in this process. A few proteins have been implicated directly in regulating this process. Oversynthesis of Pex11p leads to the formation of small peroxisomes, whereas cells lacking Pex11p have fewer but larger peroxisomes than normal (Marshall *et al*., 1995; Sakai *et al*., 1995; Li and Gould, 2002; Li *et al*., 2002). The dynamin-related protein Vps1p (Hoepfner *et al*., 2001) and

Table 1. *S. cerevisiae* strains used in this study

Strain	Genotype	Derivation
BY4742	*MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0*	Giaever *et al*., 2002
DF5α	*MATα, ura3-52, his3-200, trp1-1, leu2-3, 112, lys2-801*	Finley *et al*., 1987
DF5a	*MATa, ura3-52, his3-200, trp1-1, leu2-3, 112, lys2-801*	Finley *et al*., 1987
SFY526	*MATα, ura3-52, his3-200, ade2-101, lys2-801, trp1-901, leu2-3, 112, gal4-542, gal80-538, LYS2::GAL1*$_{UAS}$*-GAL1*$_{TATA}$*-lacZ, MEL1*	Harper *et al*., 1993
yor193Δ	*MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, yor193w::KanMX4*	Giaever *et al*., 2002
pex25Δ	*MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, pex25::KanMX4*	Giaever *et al*., 2002
pex11Δ	*MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, pex11::KanMX4*	Giaever *et al*., 2002
yor193Δ/pex25Δ	*MATa, his3Δ1, leu2Δ0, ura3Δ0, yor193w::KanMX4, pex25::KanMX4*	This study
yor193Δ/pex11Δ	*MATα, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, yor193w::KanMX4, pex11::KanMX4*	This study
pex25Δ/pex11Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, pex25::KanMX4, pex11::KanMX4*	This study
yor193Δ-HD	*MATa/MATα, his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/+, +/lys2Δ0, ura3Δ0/ura3Δ0, yor193w::KanMX4*	Giaever *et al*., 2002
pex25Δ-HD	*MATa/MATα, his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/+, +/lys2Δ0, ura3Δ0/ura3Δ0, pex25::KanMX4*	Giaever *et al*., 2002
yor193Δ-A	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, yor193w::KanMX4*	This study
pex25Δ-A	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, pex25::KanMX4*	This study
193-25-HetD	*MATa/MATα, his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/+, +/lys2Δ0, ura3Δ0/ura3Δ0, yor193w::KanMX4/+, +/pex25::KanMX4*	This study
193-11-HetD	*MATa/MATα, his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/+, +/lys2Δ0, ura3Δ0/ura3Δ0, yor193w::KanMX4/+, +/pex11::KanMX4*	This study
25-11-HetD	*MATa/MATα, his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/+, +/lys2Δ0, ura3Δ0/ura3Δ0, pex25::KanMX4/+, +/pex11::KanMX4*	This study
YOR193w-pA	*MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, yor193w::YOR193w-protA (HIS5)*	This study
PXA1-pA	*MATα, his3Δ1, leu2Δ0, lys2Δ0, met15Δ0, ura3Δ0, pxa1::PXA1-protA (HIS5)*	This study
PEX17-pA	*MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, pex17::PEX17-protA (HIS5)*	Vizeacoumar *et al*., 2003

the peroxin Pex25p (Smith *et al*., 2002) of the yeast S*accharomyces cerevisiae* have recently been shown also to be required for the control of peroxisome size and number. Cells deleted for the *VPS1* or *PEX25* gene contain enlarged peroxisomes (Hoepfner *et al*., 2001; Smith *et al*., 2002).

S. cerevisiae has been used extensively in the search for novel genes involved in peroxisome assembly. Completion of the sequence of its genome, combined with advances in microarray technology and bioinformatics, have permitted the use of global biology approaches to the identification and characterization of novel genes involved in peroxisome biogenesis in *S. cerevisiae*. Recently, transcriptome profiling of *S. cerevisiae* cells under conditions of peroxisome repression and peroxisome proliferation led to the identification of a novel gene required for peroxisome biogenesis, *PEX25* (Smith *et al*., 2002). A search of the Yeast Proteome Database revealed that Pex25p is related to a protein of unknown function encoded by the open reading frame (ORF) *YOR193w* of the *S. cerevisiae* genome. Herein, we report that Yor193p is a peripheral peroxisomal membrane protein that interacts with itself and Pex25p and functions in controlling peroxisome division and size.

MATERIALS AND METHODS

Strains and Culture Conditions

The *S. cerevisiae* strains used in this study are listed in Table 1. All strains were cultured at 30°C. Strains containing plasmids were cultured in synthetic minimal (SM) medium. Media components were as follows: YPD, 1% yeast extract, 2% peptone, 2% glucose; YPBO, 0.3% yeast extract, 0.5% peptone, 0.5% K_2HPO_4, 0.5% KH_2PO_4, 0.2% (wt/vol) Tween 40, 0.1% (vol/vol) oleic acid; SM, 0.67% yeast nitrogen base without amino acids, 2% glucose, 1× complete supplement mixture (Bio 101, Vista, CA) without histidine, uracil, leucine, or tryptophan; sporulation medium, 1% potassium acetate, 0.1% yeast extract, 0.05% glucose; YNBD, 0.67% yeast nitrogen base without amino acids, 2% glucose, supplemented with histidine, leucine, or uracil, each at 50 μg/ml.

Construction of Haploid Strains Deleted for YOR193w and PEX25, YOR193w and PEX11, and PEX25 and PEX11

The homozygous deletion diploid strains *yor193Δ*-HD and *pex25Δ*-HD were sporulated, and tetrads were dissected to select for the haploid *MATa* strains *yor193Δ*-A and *pex25Δ*-A. These strains were mated to the haploid *MATα* strains *pex25Δ* and *pex11Δ* by replica plating to obtain three heterozygous diploid strains harboring deletions for *YOR193w* and *PEX25*, *YOR193w* and *PEX11*, and *PEX25* and *PEX11*. The heterozygous diploid strains were sporulated, and tetrads from 16 heterozygous diploids were dissected for each gene deletion pair. All spores were grown in YPD medium, and DNA was extracted. Haploid strains harboring deletions in the *YOR193w* and *PEX25* genes, the *YOR193w* and *PEX11* genes, and the *PEX25* and *PEX11* genes were confirmed by polymerase chain reaction (PCR).

Protein A-tagging of Candidate Genes

Genes were genomically tagged with the sequence encoding *Staphylococcus aureus* protein A by homologous recombination with PCR-based integrative transformation into parental *BY4742* haploid cells (Aitchison *et al*., 1995; Dilworth *et al*., 2001).

Plasmids

The plasmids pDsRed-PTS1 (Smith *et al*., 2002) and pProtA/HIS5 (Rout *et al*., 2000) have been described previously. Genes to be overexpressed were amplified by PCR and cloned into the plasmid YEp13 (Broach *et al*., 1979). For overexpression, the *YOR193w* gene included 770 base pairs of upstream and 309 base pairs of downstream sequence, the *PEX11* gene included 599 base pairs of upstream and 329 base pairs of downstream sequence, and the *PEX25* gene included 753 base pairs of upstream and 319 base pairs of downstream sequence. The plasmids pGAD424 and pGBT9 (Bartel *et al*., 1993) were used for two-hybrid analysis.

Two-Hybrid Analysis

Physical interactions between Yor193p, Pex25p, and Pex11p were detected using the Matchmaker two-hybrid system (BD Biosciences Clontech, Palo Alto, CA). Chimeric genes were generated by amplifying the ORFs of the *YOR193w*, *PEX11*, and *PEX25* genes by PCR and ligating them in-frame and downstream of the DNA encoding the transcription-activating domain (AD) and the DNA-binding domain (DB) of the GAL4 transcriptional activator in the plasmids pGAD424 and pGBT9, respectively. Cells of *S. cerevisiae* strain *SFY526* were transformed simultaneously with a pGAD424-derived plasmid and a pGBT9-derived plasmid. Transformants were grown on SM medium

lacking tryptophan and leucine and tested for activation of the integrated *lacZ* construct by using β-galactosidase filter and liquid assays (Smith and Rachubinski, 2001).

Microscopy

Strains encoding protein A chimeras and transformed with the plasmid pDsRed-PTS1 were grown to mid-log phase in SM medium and then incubated in YPBO medium for 8 h. Cells were processed for immunofluorescence microscopy as described previously (Pringle et al., 1991; Tam and Rachubinski, 2002). Protein A chimeras were detected with rabbit antiserum to mouse IgG (ICN Pharmaceuticals Biochemicals Division, Aurora, OH) and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG. Images were captured on a LSM510 META (Carl Zeiss, Jena, Germany) laser scanning microscope.

Electron microscopy of whole yeast cells was performed as described previously (Goodman *et al*., 1990; Eitzen *et al*., 1997).

Morphometric Analysis of Peroxisomes

For each strain analyzed, electron images of 100 randomly selected cells were captured with a digital camera (Soft Imaging System, Lakewood, CO), and the areas of individual cells and of individual peroxisomes were determined by the program analySIS 3.1 (Soft Imaging System). To determine the average area of a peroxisome, the total peroxisome area was calculated and divided by the total number of peroxisomes counted. To quantify peroxisome number, the numerical density of peroxisomes (number of peroxisomes per cubic micrometer of cell volume) was calculated by the method described previously for spherical organelles (Weibel and Bolender 1973). Briefly, the total number of peroxisome profiles was counted and reported as the number of peroxisomes per cell area assayed (N_A). The peroxisome volume density (V_V) was then calculated as (total peroxisome area/total cell area assayed). Using the values of N_A and V_V, the numerical density of peroxisomes was determined.

Subcellular Fractionation and Isolation of Peroxisomes

Subcellular fractionation and peroxisome isolation were done essentially as described previously (Bonifacino *et al*., 2000; Smith *et al*., 2002). Cells grown overnight in YPD medium were transferred to YPBO medium and incubated for 8 h. Cells were harvested and converted to spheroplasts by digestion with Zymolyase 100T (1 mg/g of cells) in 50 mM potassium phosphate, pH 7.5, 1.2 M sorbitol, 1 mM EDTA for 1 h at 30°C. Spheroplasts were lysed by homogenization in buffer H (0.6 M sorbitol, 2.5 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 5.5, 1 mM EDTA) containing 1× complete protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN). The homogenate was subjected to centrifugation for 10 min at 2000 × *g* to yield a postnuclear supernatant (PNS) fraction. The PNS fraction was subjected to further differential centrifugation at 20,000 × *g* for 35 min to yield a pellet (20KgP) fraction enriched for peroxisomes and mitochondria and a supernatant (20KgS) fraction enriched for cytosol. The 20KgP fraction was resuspended in buffer H containing 11% Nycodenz and 1× complete protease inhibitor cocktail, and a volume containing 5 mg of protein was overlaid onto either a 30-ml continuous gradient of 30–60% (wt/vol) Nycodenz or a 30-ml discontinuous gradient consisting of 17, 25, 35, and 50% (wt/vol) Nycodenz, both in buffer H containing 1× complete protease inhibitor cocktail. Organelles were separated by centrifugation at 100,000 × *g* for 90 min in a VTi50 rotor (Beckman Coulter, Fullerton, CA). Fractions of 2 ml were collected from the bottom of the gradient.

Extraction of Peroxisomes

Peroxisomes were extracted as described previously (Fujiki *et al*., 1982; Nuttley *et al*., 1990). Essentially, organelles in the 20KgP fraction (50 μg of protein) were lysed by incubation in 10 volumes of ice-cold Ti8 buffer (10 mM Tris-HCl, pH 8.0) containing 2× complete protease inhibitor cocktail on ice for 1 h and separated by centrifugation at 200,000 × *g* for 1 h at 4°C in a TLA 120.2 rotor (Beckman Coulter) into pellet (Ti8P) and supernatant (Ti8S) fractions. The Ti8P fraction was resuspended in Ti8 buffer, and a portion was extracted with 0.1 M Na_2CO_3, pH 11.3, for 45 min on ice and then separated by centrifugation at 200,000 × *g* for 1 h at 4°C in a TLA 120.2 rotor into pellet (CO_3P) and supernatant (CO_3S) fractions. Proteins in fractions were precipitated by addition of trichloroacetic acid, and precipitates were washed with acetone. Proteins in equal portions of each fraction were separated by SDS-PAGE and analyzed by immunoblotting.

Antibodies

Antibodies to the carboxyl-terminal SKL tripeptide (Aitchison *et al*., 1992), thiolase (Eitzen *et al*., 1996), and Sdh2p (Dibrov *et al*., 1998) have been described previously. Rabbit antibodies to glucose-6-phosphate dehydrogenase (G6PDH) of *S. cerevisiae* were obtained from Sigma-Aldrich (St. Louis, MO). Horseradish peroxidase-conjugated donkey anti-rabbit IgG and horseradish peroxidase-conjugated goat anti-guinea pig IgG secondary antibodies (Amersham Biosciences, Piscataway, NJ) were used to detect primary antibodies in immunoblot analysis. Fluorescein isothiocyanate-conjugated anti-rabbit IgG and rhodamine-conjugated anti-guinea pig IgG (Jackson Immunoresearch Laboratories, West Grove, PA) were used to detect primary antibodies in immunofluorescence microscopy.

Analytical Procedures

Whole cell lysates were prepared as described previously (Eitzen *et al*., 1997). Extraction of nucleic acid from yeast lysates and manipulation of DNA were performed as described previously (Ausubel *et al*., 1994). Immunoblotting was performed using a wet transfer system (Ausubel *et al*., 1994), and antigen-antibody complexes in immunoblots were detected by enhanced chemiluminescence (Amersham Biosciences). Protein concentration was determined using a commercially available kit (Bio-Rad, Hercules, CA) and bovine serum albumin as a standard.

RESULTS

Yor193p Exhibits Extensive Sequence Similarity to Pex11p and Pex25p

Pex25p, a novel peripheral peroxisomal membrane protein identified by transcriptome profiling, has been shown to be required for the regulation of peroxisome size and maintenance in *S. cerevisiae* (Smith *et al*., 2002). A search of the Yeast Proteome Database (https://www.incyte.com/proteome/databases.jsp) showed that a protein of unknown function encoded by the hypothetical ORF, *YOR193w*, of the *S. cerevisiae* genome shares extensive sequence similarity to Pex25p (19.5% identical amino acids, 25.9% similar amino acids; Figure 1). Pex25p has been reported to show similarity also to Pex11p (Smith *et al*., 2002) (10.9% identical amino acids, 19.0% similar amino acids; Figure 1), and likewise Yor193p shows similarity to Pex11p (9.3% identical amino acids, 18.4% similar amino acids; Figure 1). Yor193p is predicted to be a protein 376 amino acids in length with a molecular weight of 44,149. Yor193p has no predicted transmembrane domain (http://www.cbs.dtu.dk/services/THMMM-2.0/) (Krogh *et al*., 2001).

Cells Deleted for One or Two of the YOR193w, PEX25, and PEX11 Genes Contain Enlarged Peroxisomes

Yeast strains harboring individual deletions of the *YOR193w*, *PEX25*, and *PEX11* genes, or double deletions of the *YOR193w* and *PEX25*, *YOR193w* and *PEX11*, and *PEX25* and *PEX11*genes were assayed for growth in the presence of glucose or oleic acid as the sole carbon source. All strains grew comparably on glucose-containing YPD medium (our unpublished data). As expected, cells deleted for the *PEX3* gene, which lack functional peroxisomes (Höhfeld *et al*., 1991; Hettema *et al*., 2000), failed to grow on oleic acid-containing YPBO medium. Although cells deleted for *YOR193w* or *PEX25* grew on containing YPBO medium at a rate like or very similar to that of wild-type *BY4742* cells, cells deleted for both the *YOR193w* and *PEX25* genes displayed a growth defect on YPBO medium, suggesting that Yor193p and Pex25p together influence the rate of growth on oleic acid-containing medium (Figure 2). Cells deleted for the *YOR193w* and *PEX11* genes or the *PEX25* and *PEX11* genes showed a similar growth defect on YPBO medium as did cells deleted for *PEX11* alone, suggesting that Pex11p plays the dominant role among these three proteins in growth on oleic acid medium (Figure 2).

Immunofluorescence analysis of oleic acid-incubated wild-type *BY4742* cells with antibodies to the carboxyl-terminal PTS1 tripeptide Ser-Lys-Leu (SKL) or to the PTS2-containing enzyme thiolase (THI) showed a pattern of numerous small punctate structures characteristic of peroxisomes (Figure 3). In contrast, the majority of cells of the *yor193*Δ, *pex25*Δ, *pex11*Δ, *yor193*Δ/pex25Δ, *yor193*Δ/pex11Δ, and pex25Δ/pex11Δ strains stained with the same

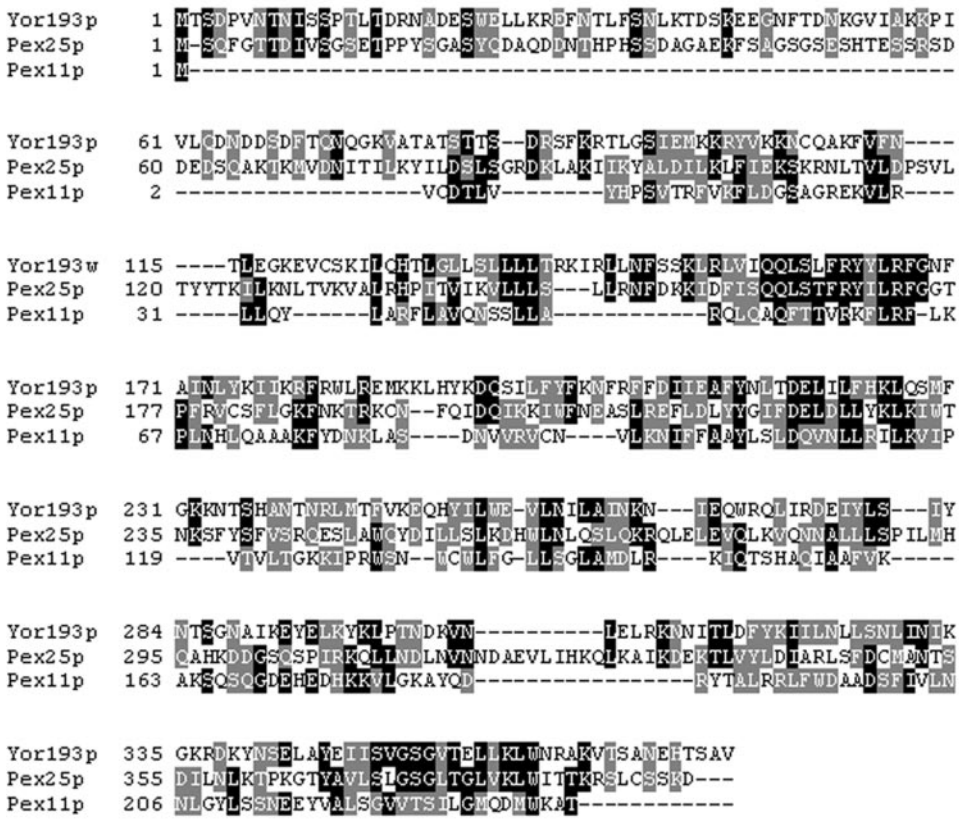

Figure 1. Sequence alignment of Yor193p, Pex25p, and Pex11p. Amino acid sequences were aligned with the use of the ClustalW program (EMBL-EBI, Cambridge, United Kingdom) (http://www.ebi.ac.uk.clustalw/). Identical residues (black) and similar residues (gray) in at least two of the proteins are shaded. Similarity rules: G = A = S; A = V; V = I = L = M; I = L = M = F = Y = W; K = R = H; D = E = Q = N; and S = T = Q = N. Dashes represent gaps.

antibodies showed one or two large peroxisomes per cell (Figure 3). In electron micrographs, wild-type cells grown in oleic acid-containing medium contained characteristic peroxisomes 0.2 to 0.4 μm in diameter (Figure 4A). In contrast, cells of the *yor193*Δ, *pex25*Δ, and *pex11*Δ strains contained enlarged peroxisomes (Figure 4, B–D). The peroxisomes were even larger in *yor193*Δ/pex25Δ, *yor193*Δ/pex11Δ, and *pex25*Δ/pex11Δ cells (Figure 4, E–G). Morphometric analysis showed that cells harboring double gene deletions contained less peroxisomes than did wild-type cells or cells deleted for one of the *YOR193w*, *PEX25*, and *PEX11* genes and that, on average, these peroxisomes were much larger (Table 2). Cells of all deletion strains contained much greater numbers of peroxisomes with areas of 0.15 μm^2 or larger than did wild-type cells (Figure 4H). Cells harboring double deletions of the *YOR193w*

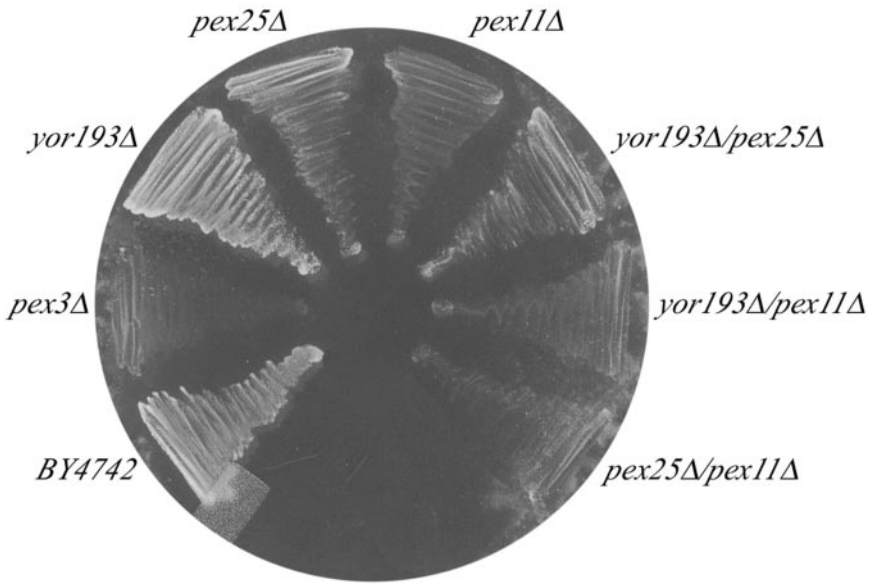

Figure 2. Growth of various strains on oleic acid-containing (YPBO) medium. All strains were grown on YPBO agar for 4 d at 30°C.

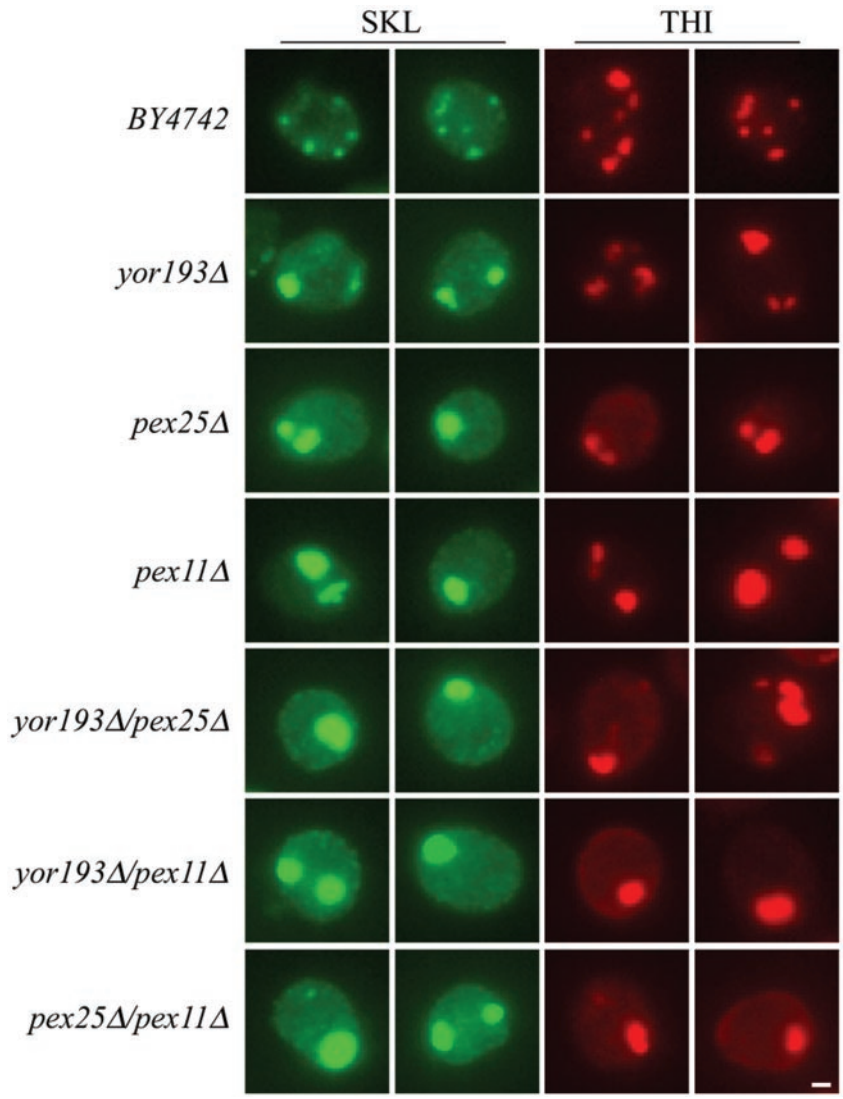

Figure 3. Peroxisomes are enlarged in cells deleted for one or two of the *YOR193w*, *PEX25*, and *PEX11* genes. Wild-type *BY4742* cells and cells of the *yor193*Δ, *pex25*Δ, *pex11*Δ, *yor193*Δ/pex25Δ, *yor193*Δ/pex11Δ, and *pex25*Δ/pex11Δ deletion strains were grown in YPD medium for 16 h, transferred to YPBO medium, and incubated for 8 h in YPBO medium. Cells were processed for immunofluorescence microscopy with antibodies to the PTS1 tripeptide SKL or to the PTS2-containing protein THI. Rabbit primary antibodies (SKL) were detected with fluorescein-conjugated secondary antibodies. Guinea pig primary antibodies (THI) were detected with rhodamine-conjugated secondary antibodies. Bar, 1 μm.

and *PEX25*, the *YOR193w* and *PEX11*, and the *PEX25* and *PEX11* genes contained greatly enlarged peroxisomes with areas of ≥ 0.6 μm^2. Peroxisomes of these sizes were not observed in wild-type cells or cells deleted for any one of the *YOR193w*, *PEX25*, and *PEX11* genes.

Nycodenz density gradient centrifugation analysis showed that peroxisomes isolated from all deletion strains have peak densities (fraction 4, 1.2328 g/cm^3, in *pex11*Δ and *pex25*Δ/pex11Δ cells; fraction 5, 1.221 g/cm^3, in *yor193*Δ and *yor193*Δ/pex11Δ cells; fraction 6, 1.2106 g/cm^3, in *pex25*Δ and *yor193*Δ/pex25Δ cells) greater than that of peroxisomes isolated from wild-type cells (fraction 7, 1.2077 g/cm^3) (Figure 5).

Yor193p Is a Peripheral Membrane Protein of Peroxisomes

A fluorescent chimera between *Discosoma* sp. red fluorescent protein (DsRed) and the PTS1 Ser-Lys-Leu targets to peroxisomes of *S. cerevisiae* (Smith *et al.*, 2002; Vizeacoumar *et al.*, 2003). Genomically encoded protein A chimeras of Yor193p and the peroxisomal peroxin Pex17p, which act like their wild-type counterparts in functionally complementing their respective gene deletion mutant strains (our unpublished data), were localized in oleic acid-incubated cells by indirect immunofluorescence microscopy combined with direct fluorescence microscopy to identify peroxisomes. Yor193-pA and Pex17-pA colocalized with DsRed-PTS1 to punctuate structures characteristic of peroxisomes by confocal microscopy (Figure 6A). Subcellular fractionation was also used to establish whether Yor193p is associated with peroxisomes. Yor193-pA, like the peroxisomal matrix protein thiolase, localized preferentially to the 20KgP fraction enriched for peroxisomes and mitochondria (Figure 6B). Isopycnic density gradient centrifugation of the 20KgP fraction showed that Yor193p cofractionated with thiolase but not with the mitochondrial protein, Sdh2p (Figure 6C). Therefore, both confocal microscopy and subcellular fractionation showed Yor193p to be a peroxisomal protein.

Organelle extraction was used to determine the suborganellar location of Yor193p. Peroxisomes were hypotonically lysed in dilute alkali Tris buffer and subjected to ultracentrifugation to yield a supernatant (Ti8S) fraction enriched for matrix proteins and a pellet (Ti8P) fraction enriched for membrane proteins (Figure 6D). Yor193-pA cofractionated with the protein A chimeras of the periph-

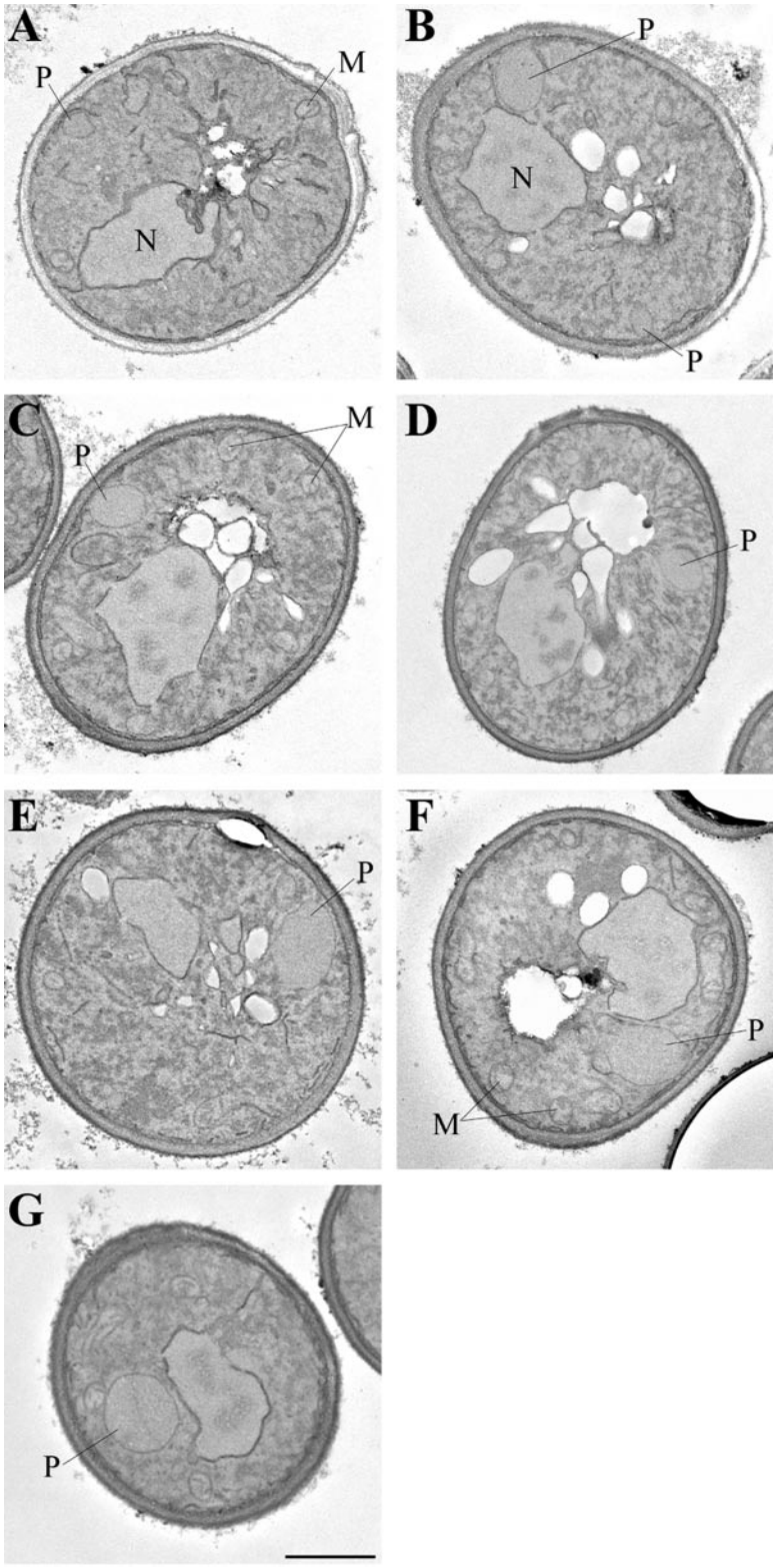

Figure 4. Cells harboring double deletions of *YOR193w* and *PEX25*, *YOR193w* and *PEX11*, and *PEX25* and *PEX11* contain greatly enlarged peroxisomes. Ultrastructure of wild-type *BY4742* (A), *yor193*Δ (B), *pex25*Δ (C), *pex11*Δ (D), *yor193*Δ/pex25Δ (E), *yor193*Δ/pex11Δ (F), and *pex25*Δ/pex11Δ (G) cells. Cells were grown in YPD medium for 16 h, shifted to YPBO medium, and incubated in YPBO medium for an additional 8 h. Cells were fixed in 3% $KMnO_4$ and proceed for electron microscopy. P, peroxisome; M, mitochondrion; N, nucleus. Bar, 1 μm. (H) Morphometric analysis of peroxisomes. For each strain analyzed, the areas of individual peroxisomes of 100 randomly selected cells were determined using the program analySIS 3.1. Peroxisomes were then separated into size categories. A histogram was generated for each strain depicting the percentage of total peroxisomes occupied by the peroxisomes of each category. The numbers along the *x*-axis are the maximum sizes of peroxisomes (in square micrometers) in each category, with the exception of the last number, which represents the minimum size of peroxisomes (in square micrometers) in the last category.

eral peroxisomal membrane protein Pex17p (Huhse *et al*., 1998; Vizeacoumar *et al*., 2003) and the integral peroxisomal membrane protein Pxa1p (Swartzman *et al*., 1996) to the Ti8P fraction. The soluble peroxisomal matrix protein thiolase was found almost exclusively in the Ti8S fraction. The Ti8P fractions were then extracted with alkali Na_2CO_3 and subjected to ultracentrifugation. This treatment releases proteins associated with, but not integral to, membranes (Fujiki *et al*., 1982). Yor193-pA cofractionated with Pex17-pA to the supernatant (CO_3S) fraction enriched for

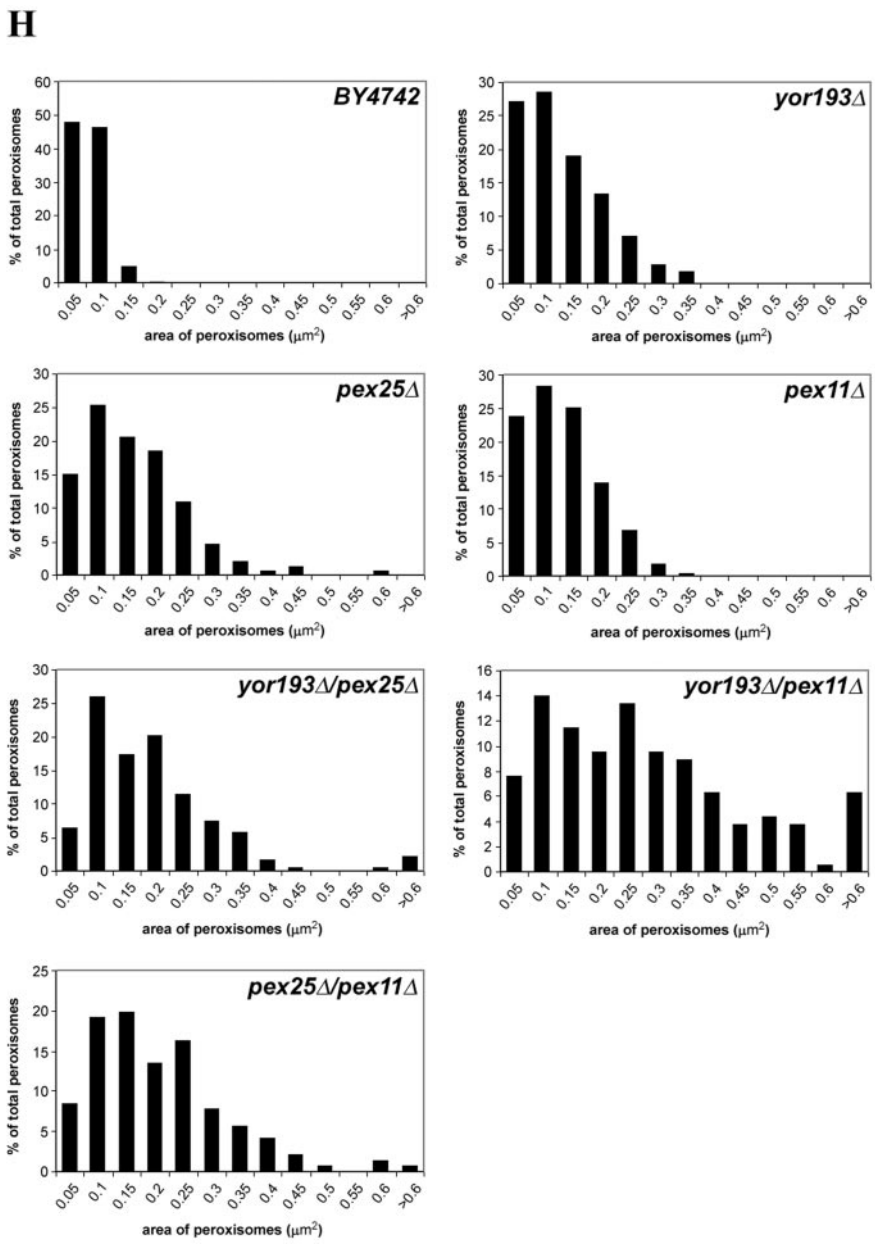

Figure 4 Continued.

Table 2. Average area and numerical density of peroxisomes in cells of wild-type and deletion strains

Strain	Cell area assayed (μm^2)	Peroxisome count[a]	Numerical density of peroxisomes[b]	Average area of peroxisomes[c] (μm^2)
BY4742	853	0.21	0.98	0.051
*yor193*Δ	909	0.23	0.77	0.088
*pex25*Δ	892	0.16	0.48	0.113
*pex11*Δ	918	0.24	0.81	0.097
*yor193*Δ/*pex25*Δ	1114	0.16	0.30	0.152
*yor193*Δ/*pex11*Δ	999	0.16	0.33	0.224
*pex25*Δ/*pex11*Δ	914	0.15	0.39	0.158

[a] Number of peroxisomes counted per square micrometer of cell area on micrographs.

[b] Number of peroxisomes per cubic micrometer of cell volume (Weibel and Bolender, 1973).

[c] Average area on micrographs.

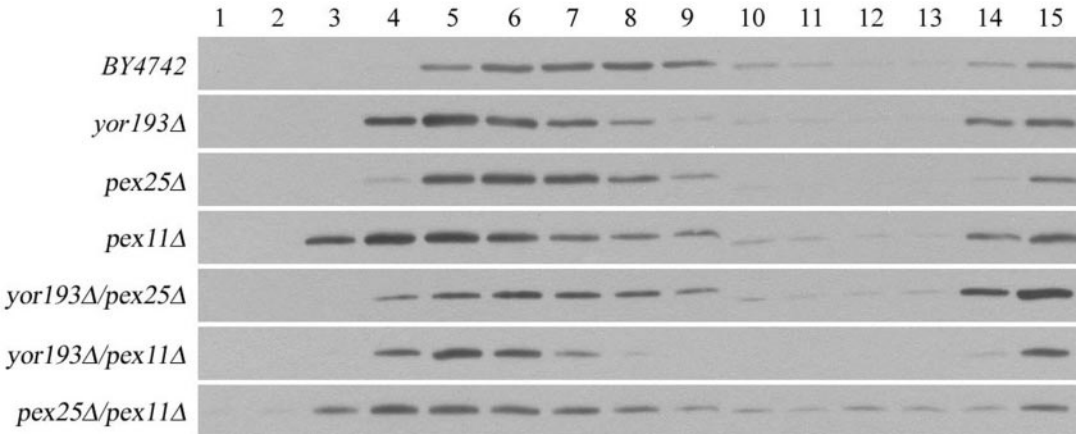

Figure 5. Peroxisomes isolated from cells deleted for one or two of the *YOR193w*, *PEX25*, and *PEX11* genes are more dense than isolated wild-type peroxisomes. The wild-type strain *BY4742* and the deletion strains *yor193*Δ, *pex25*Δ, *pex11*Δ, *yor193*Δ/pex25Δ, *yor193*Δ/pex11Δ, and *pex25*Δ/pex11Δ were grown overnight in YPD medium, transferred to YPBO medium, and incubated in YPBO medium for 8 h. A PNS fraction was prepared from cells of each strain and divided by centrifugation into 20KgS and 20KgP fractions. Organelles in the 20KgP fraction were separated by isopycnic centrifugation on a continuous 30–60% Nycodenz gradient. Fifteen 2-ml fractions were collected from the bottom of each gradient. Equal volumes of each fraction were analyzed by immunoblotting with antibodies to the peroxisomal matrix enzyme thiolase to detect peroxisomes.

peripheral membrane proteins (Figure 6D). In contrast, Pxa1-pA fractionated to the pellet (CO_3P) fraction enriched for integral membrane proteins. Therefore, these data suggest that Yor193p is a peripheral membrane protein of peroxisomes, as has been shown for Pex25p (Smith *et al*., 2002) and Pex11p (Marshall *et al*., 1995).

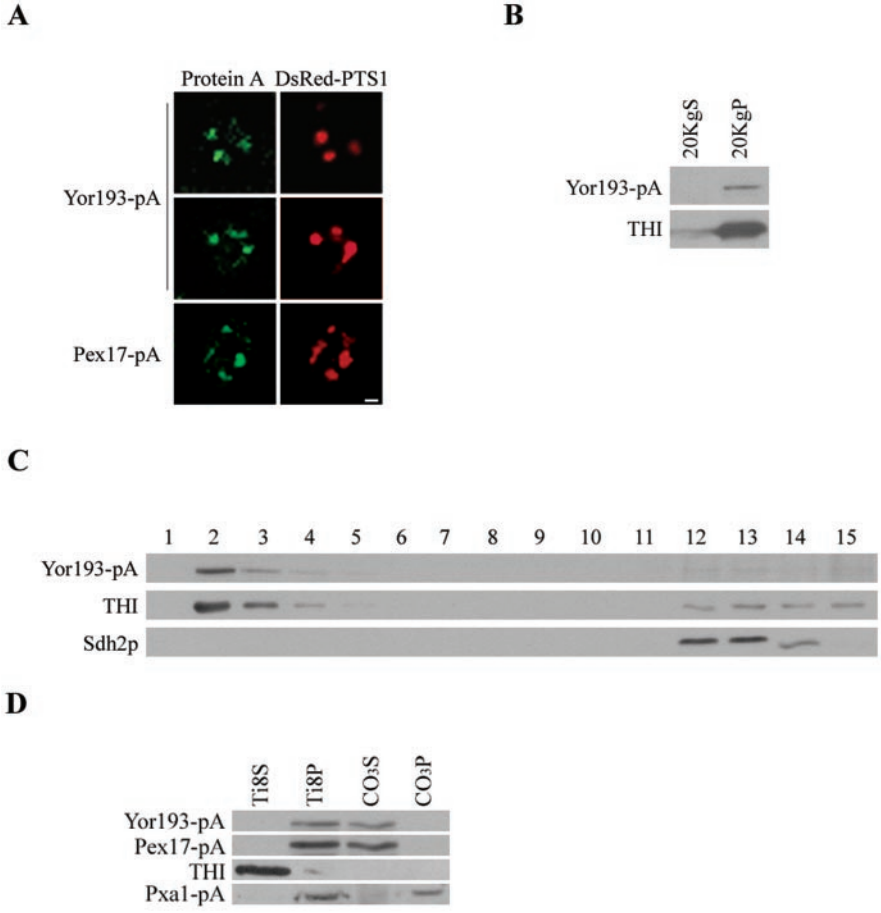

Figure 6. Yor193-pA is a peripheral peroxisomal membrane protein. (A) Yor193-pA colocalizes with DsRed-PTS1 in punctate structures characteristic of peroxisomes by double labeling, indirect immunofluorescence microscopy. Bar, 1 μm. (B) Yor193-pA localizes to the 20KgP subcellular fraction enriched for peroxisomes. Immunoblot analysis of the 20KgS and 20KgP subcellular fractions from cells expressing Yor193-pA was performed with antibodies to THI. (C) Yor193-pA cofractionates with peroxisomes. Organelles in the 20KgP fraction were separated by isopycnic centrifugation on a discontinuous Nycodenz gradient. Fractions were collected from the bottom of the gradient, and equal portions of each fraction were analyzed by immunoblotting. Fractions enriched for peroxisomes and mitochondria were identified by immunodetection of thiolase and Sdh2p, respectively. (D) The 20KgP fraction from cells expressing Yor193p-pA, Pex17-pA, or Pxa1-pA was treated with 10 mM Tris-HCl, pH 8.0, to lyse peroxisomes and then subjected to centrifugation to yield a supernatant (Ti8S) fraction enriched for matrix proteins and a pellet (Ti8P) fraction enriched for membrane proteins. The Ti8P fractions were further treated with 0.1 M Na_2CO_3, pH 11.3, and separated by centrifugation into a supernatant (CO_3S) fraction enriched for peripherally associated membrane proteins and a pellet (CO_3P) fraction enriched for integral membrane proteins. Equal portions of each fraction were analyzed by immunoblotting. Immunodetection of thiolase, Pex17-pA and Pxa1-pA marked the fractionation profiles of a peroxisomal matrix, peripheral membrane, and integral membrane protein, respectively.

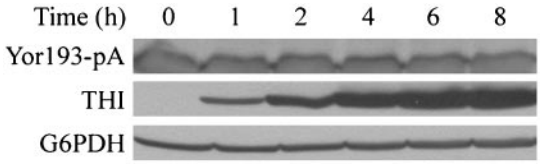

Figure 7. Synthesis of Yor193-pA remains constant during incubation of *S. cerevisiae* in oleic acid-containing medium. Cells grown for 16 h in YPD medium were shifted to, and incubated in, YPBO medium. Aliquots of cells were removed from the YPBO medium at the times indicated, and total cell lysates were prepared. Equal amounts of protein from the total cell lysates were separated by SDS-PAGE and analyzed by immunoblotting with antibodies to thiolase to visualize the protein A fusion and thiolase. Antibodies directed against G6PDH were used to confirm the loading of equal amount of proteins in each lane.

Synthesis of Yor193p Remains Constant during Incubation of Cells in Oleic Acid-containing Medium

The synthesis of many peroxisomal proteins is induced by incubating yeast cells in medium containing oleic acid. The genomically encoded protein A chimera of Yor193p was analyzed to monitor the expression of *YOR193w* under the control of its endogenous promoter. Cells synthesizing Yor193-pA were grown in glucose-containing YPD medium and transferred to oleic acid-containing YPBO medium. Aliquots of cells were removed at various times after the transfer to YPBO medium, and their lysates were subjected to SDS-PAGE and immunoblotting (Figure 7). Yor193-pA was detected in YPD medium at the time of transfer, and its level remained unchanged during incubation in YPBO. Under the same conditions, the level of the peroxisomal matrix enzyme THI increased dramatically from undetectable levels with time of incubation in YPBO, whereas the level of the cytosolic enzyme G6PDH remained constant and acted as a control for protein loading.

Physical Interactions between Yor193p, Pex25p, and Pex11p

A limited yeast two-hybrid screen was performed to identify physical interactions between Yor193p, Pex25p, and Pex11p. Others have used this methodology to detect interactions between peroxins (for examples, see Girzalsky *et al*., 1999; Smith and Rachubinski, 2001; Sichting *et al*., 2003). Chimeric genes were made by ligating the ORFs of *YOR193w*, *PEX25* and *PEX11* in frame and downstream of sequences encoding one of the two functional domains (AD or DB) of the GAL4 transcriptional activator. All possible combinations of plasmid pairs encoding AD and DB fusion proteins were transformed into *S. cerevisiae* strain *SYF526*, and initially β-galactosidase filter detection assays were performed. An interaction was detected between Yor193p and Pex25p (Figure 8A). An interaction was also detected between Pex11p and itself (Figure 8A), which was expected because Pex11p has been shown previously to form homodimers (Marshall *et al*., 1996). Potential interactions were also detected between Pex25p and itself, and between Yor193p and itself, by using the filter detection assay (our unpublished data).

To confirm the results of the filter detection assay, liquid β-galactosidase assays were performed (Figure 8B). Cell lysates of strains synthesizing both Yor193p and Pex25p, Pex11p and Pex11p, Pex25p and Pex25p, and Yor193p and

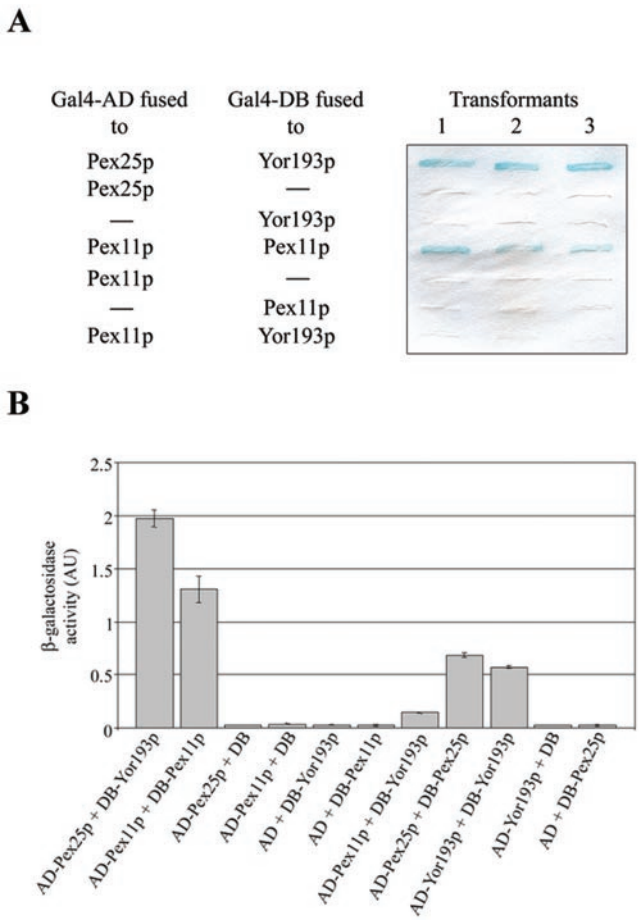

Figure 8. Analysis of interactions between Yor193p, Pex11p, and Pex25p by the yeast two-hybrid system. (A) β-Galactosidase filter detection assay. *SFY526* cells synthesizing both Gal4-AD (left) and Gal4-DB (right) fusion proteins were tested for β-galactosidase activity. The color intensities of three independent transformants for each strain are shown. (B) β-Galactosidase liquid culture assay. A comparison of β-galactosidase activities of strains doubly transformed with plasmids encoding the designated fusion or fusions (*x*-axis). β-Galactosidase activity is measured in arbitrary units (AU) as defined by the manufacturer (BD Biosciences Clontech). Each bar reports the average β-galactosidase activity of three individual transformants ± SD.

Yor193p fusion proteins showed greater β-galactosidase activity than lysates of control strains synthesizing either one or the other of the fusion proteins. These results suggest that Yor193p and Pex25p interact physically and that Pex11p, Pex25p, and Yor193p interact with themselves. Further experimentation is required to determine whether these interactions are direct or bridged by other proteins.

Overexpression of YOR193w, PEX25, or PEX11 Promotes Peroxisome Division

The wild-type strain *BY4742* and strains deleted for one or two of the genes *YOR193w*, *PEX25*, and *PEX11* were transformed with multicopy plasmids for overexpression of the *YOR193w*, *PEX25*, and *PEX11* genes. Transformants grown in oleic acid-containing medium were analyzed by indirect immunofluorescence microscopy with antibodies to the PTS1 SKL and to the PTS2-containing protein thiolase (Fig-

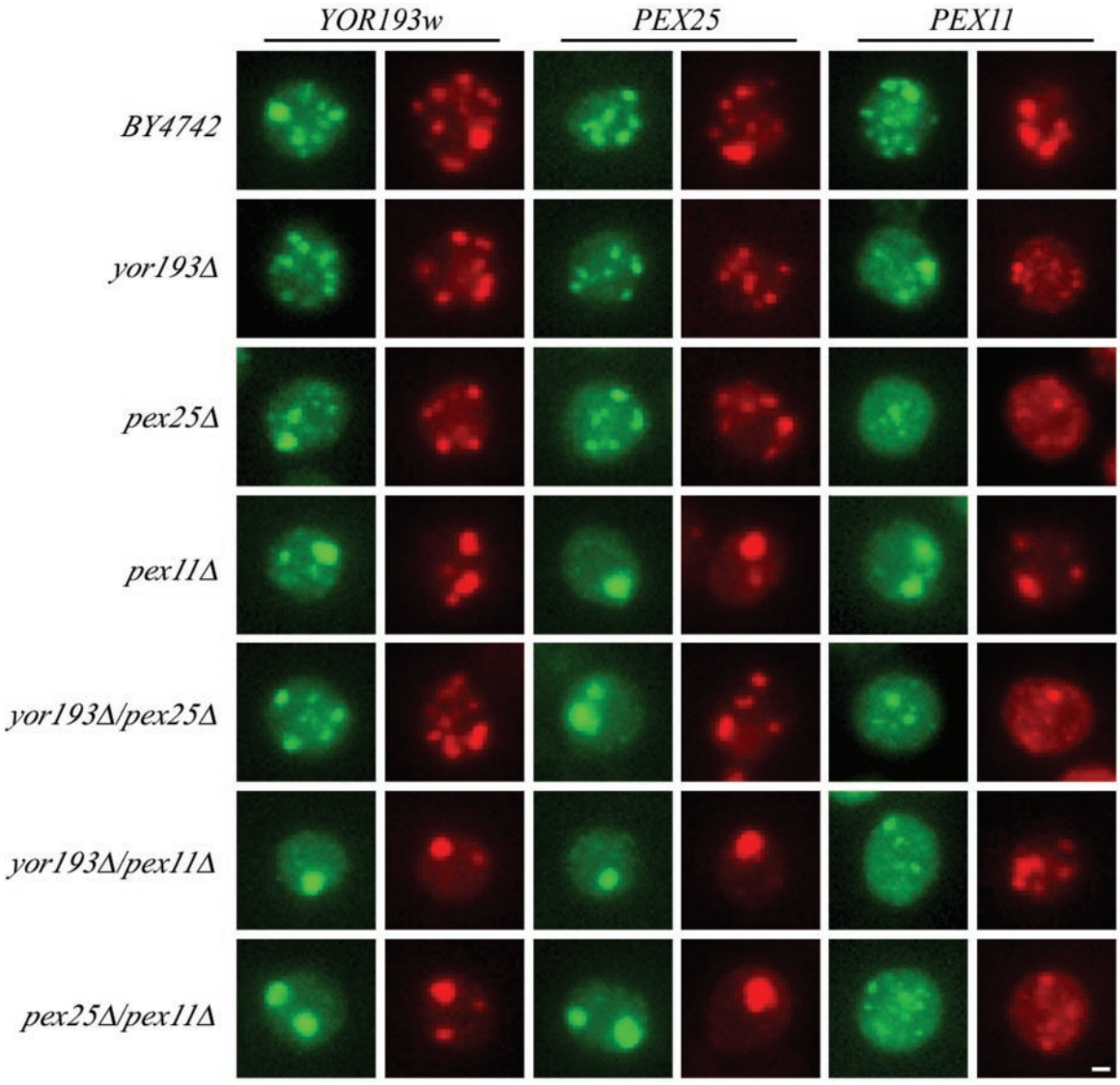

Figure 9. Overexpression of *YOR193w*, *PEX25*, and *PEX11* induces peroxisome division. Immunofluorescence microscopic analysis of overexpression of the *YOR193w*, *PEX25*, and *PEX11* genes. Cells were grown for 16 h in SM medium, transferred to YPBO medium, and incubated in YPBO medium for 8 h. The gene being overexpressed is given at the top of the figure, and the strains in which overexpression is being done are given at the left of the figure. Peroxisomes were detected by indirect immunofluorescence microscopy by using rabbit anti-SKL antibodies and guinea pig anti-thiolase antibodies, followed by fluorescein-conjugated anti-rabbit IgG secondary antibodies and rhodamine-conjugated anti-guinea pig IgG secondary antibodies. Bar, 1 μm.

ure 9) and by electron microscopy (Table 3). All transformants were able to grow on medium containing oleic acid as the sole carbon source (our unpublished data). It has been reported previously that overexpression of Pex11p promotes peroxisomal proliferation (Marshall *et al*., 1995; Li and Gould, 2002; Li *et al*., 2002). Overexpression of *PEX11* in all deletion strains led to the formation of small peroxisomes, some of which seemed to cluster, and to the almost total disappearance of the enlarged peroxisomes that were observed in the original deletion strains (Figure 9 and Table 3). Overexpression of *YOR193w* in *yor193*Δ cells restored the wild-type phenotype and led to the production of a small number of small peroxisomes (Figure 9 and Table 3). Formation of both normal and small peroxisomes was observed in *pex25*Δ and *yor193*Δ/pex25Δ cells overexpressing *YOR193w* (Figure 9 and Table 3). In contrast, overexpression of *YOR193w* had little or no effect on peroxisome size in *pex11*Δ, *yor193*Δ/pex11Δ, and *pex25*Δ/pex11Δ cells (Figure 9 and Table 3). Normal peroxisomes were observed in *yor193*Δ, *pex25*Δ, and *yor193*Δ/pex25Δ cells overexpressing *PEX25* (Figure 9 and Table 3); however, these cells also contained some large peroxisomes and clusters of peroxisomes (Table 3). No reduction in peroxisome size was observed in *pex11*Δ, *yor193*Δ/pex11Δ, and *pex25*Δ/pex11Δ overexpressing *PEX25* (Figure 9 and Table 3). It should be noted that overexpression of *YOR193w* or *PEX25* caused extensive clustering of peroxisomes in cells deleted for the *PEX11* gene (Table 3). Last, minor clustering of peroxisomes was observed in wild-type cells overexpressing *YOR193w*, *PEX25*, or *PEX11* (Table 3).

Table 3. Summary of phenotypes observed in cells overexpressing *YOR193w, PEX25,* or *PEX11*

Strain	Overexpressed gene	Normal peroxisomes	Large peroxisomes	Small peroxisomes	Clustered peroxisomes
yor193Δ	–	++[a]	+++		
yor193Δ	*YOR193w*	++++		+	
yor193Δ	*PEX25*	++++	+		
yor193Δ	*PEX11*			++++	+
pex25Δ	–	++	+++		
pex25Δ	*YOR193w*	+	+	+++	
pex25Δ	*PEX25*	++++	+		
pex25Δ	*PEX11*		+	+++	+
pex11Δ	–	+	++++		
pex11Δ	*YOR193w*		+		++++
pex11Δ	*PEX25*		++		+++
pex11Δ	*PEX11*		+	+	+++
yor193Δ/pex25Δ	–	+	++++		
yor193Δ/pex25Δ	*YOR193w*	++	+	+	+
yor193Δ/pex25Δ	*PEX25*	++	++		+
yor193Δ/pex25Δ	*PEX11*			+++	++
yor193Δ/pex11Δ	–		+++++		
yor193Δ/pex11Δ	*YOR193w*		+++++		
yor193Δ/pex11Δ	*PEX25*		+++++		
yor193Δ/pex11Δ	*PEX11*		+	++	++
pex25Δ/pex11Δ	–		+++++		
pex25Δ/pex11Δ	*YOR193w*	+	++++		
pex25Δ/pex11Δ	*PEX25*		+++++		
pex25Δ/pex11Δ	*PEX11*			+++	++
BY4742	–	+++++			
BY4742	*YOR193w*	+++			++
BY4742	*PEX25*	++	+		++
BY4742	*PEX11*	+++			++

[a] The (+) symbol denotes the presence of a particular peroxisomal phenotype. Increased numbers of (+) symbols denote increased prevalence of a particular peroxisomal morphological phenotype. The absence of a (+) symbol denotes the absence of a particular peroxisomal morphological phenotype.

DISCUSSION

Completion of the *S. cerevisiae* genome sequence has permitted the use of transcriptome profiling of cells incubated in oleic acid-containing medium versus cells incubated in glucose-containing medium to predict gene involvement in peroxisome biogenesis or function (Smith *et al*., 2002). This method led to the successful identification of a novel *PEX* gene, *PEX25*, involved in the regulation of peroxisome size and maintenance (Smith *et al*., 2002). A search of the Yeast Proteome Database revealed that Pex25p shares extensive amino acid identity and similarity to a protein of unknown function encoded by the ORF *YOR193w* of the *S. cerevisiae* genome. Yor193p also shows a great degree of amino acid identity and similarity to another previously characterized peroxisomal protein, Pex11p. A genomically encoded protein A chimera of Yor193p localizes to peroxisomes and displays the characteristics of a peripheral membrane protein, as do both Pex25p and Pex11p (Marshall *et al*., 1996; Smith *et al*., 2002).

Yor193p is not required for growth of cells on oleic acid-containing medium, because *yor193Δ* cells showed a growth rate comparable with that of wild-type cells on this medium. This finding might explain why *YOR193w* was not identified as a gene required for peroxisome assembly by classical negative selection procedures involving the isolation of mutant yeast strains that fail to grow in the presence of oleic acid as sole carbon source. Also, synthesis of Yor193p remains constant during growth of cells in oleic acid-containing medium, providing an explanation for why *YOR193w* was not identified by transcriptome profiling as a gene potentially involved in peroxisome assembly (Smith *et al*., 2002). Yor193p, as well as Pex25p and Pex11p, are not required for peroxisome assembly per se, because cells lacking the *YOR193w*, *PEX25*, or *PEX11* gene still contain peroxisomes. These peroxisomes are partially functional, as the cells harboring individual gene deletions could still grow on oleic acid-containing medium, although at rates slower than that of wild-type cells. However, these peroxisomes are not normal, because they are larger than wild-type peroxisomes. Peroxisomes of cells containing deletions of two of the *YOR193w*, *PEX25*, and *PEX11* genes are even larger than those of cells deleted for the individual genes. These abnormally large peroxisomes could result from a disruption of components of the peroxisome division machinery in cells of the gene deletion strains, which is consistent with a role for *YOR193w* in the control of peroxisome size, as has been proposed for *PEX25* and *PEX11* (Marshall *et al*., 1995; Erdmann and Blobel, 1995; Smith *et al*., 2002). Although the majority of the *yor193Δ*, *pex25Δ*, and *pex11Δ* cells contain enlarged peroxisomes, significant numbers of cells of these deletion strains still contain peroxisomes that are wild-type in appearance. This heterogeneity in the population of peroxisomes in cells harboring single gene deletions implies the existence of more than one checkpoint for peroxisome division. Moreover, the fact that peroxisomes are even larger in cells harboring two gene deletions suggests that Yor193p,

Pex25p, and Pex11p function additively to control peroxisome division.

Previous studies have implicated Pex11p as an effector of peroxisome division in different organisms (Marshall *et al*., 1995; Erdmann and Blobel, 1995; Sakai *et al*., 1995; Abe and Fujiki, 1998; Lorenz *et al*., 1998; Passreiter *et al*., 1998; Schrader *et al*., 1998; Li and Gould, 2002; Li *et al*., 2002). Pex25p has also been implicated in the control of peroxisome size and number in *S. cerevisiae* (Smith *et al*., 2002). So how might Yor193p, Pex25p, and Pex11p act and interact to regulate the size and number of peroxisomes in *S. cerevisiae*? To address this question, we performed a limited yeast two-hybrid screen to identify physical interactions among Yor193p, Pex25p, and Pex11p and overexpressed the genes for these proteins in cells of the wild-type strain and of the various gene deletion mutant strains to determine the effects of protein overproduction on peroxisome morphology.

Overexpression of *PEX11* in *S. cerevisiae* cells deleted for this gene has been reported to induce the formation of large numbers of small peroxisomes (Marshall *et al*., 1995). In contrast, overexpression of *YOR193w* and *PEX25* in their respective gene deletion backgrounds did not induce the production of large numbers of small peroxisomes but resulted essentially in the recovery of the wild-type peroxisomal phenotype. However, overexpression of *YOR193w* in both *yor193*Δ and *pex25*Δ cells promoted the formation of a limited amount of small peroxisomes, whereas some large peroxisomes could still be observed in cells overexpressing *PEX25*. Small peroxisomes are produced in all deletion strains overexpressing the *PEX11* gene. Therefore, Pex11p is likely to play the dominant effector role in peroxisome division, whereas Yor193p and Pex25p are secondary effectors of peroxisome division, with Yor193p being stronger than Pex25p. Interestingly, in all strains overexpressing *PEX11*, the small peroxisomes that are formed remain largely adherent. Considering peroxisome proliferation as a two-step process, namely, peroxisome division and peroxisome separation, Pex11p may be involved primarily in division with limited or no significant contribution to separation. Overexpression of *YOR193w* or *PEX25* in wild-type, *pex11*Δ or *yor193*Δ/*pex25*Δ cells led to the formation of some adherent peroxisomes, suggesting that Yor193p and Pex25p have some, but limited, effects on peroxisome separation. Other proteins are expected to influence this separation step. Indeed, we have recently reported the identification of two peroxisomal membrane proteins, Pex28p and Pex29p, required for peroxisomal separation in *S. cerevisiae*. Cells lacking Pex28p and Pex29p contain clusters of peroxisomes that often exhibit thickened membranes between adjacent peroxisomes (Vizeacoumar *et al*., 2003).

Using the yeast two-hybrid system, we identified interactions between Yor193p and Pex25p and self-interactions with Yor193p, Pex25p and Pex11p. Results from liquid β-galactosidase assays suggest that the interaction between Yor193p and Pex25p is the strongest among all detected interactions. It should be noted, however, that because the two-hybrid analysis was performed within an homologous system, i.e., within *S. cerevisiae* cells containing wild-type copies of the *YOR193w*, *PEX11*, and *PEX25* genes, the interactions observed may represent only a subset of all possible interactions because of competition from endogenous Yor193p, Pex11p, and Pex25p. No interaction was detected between Yor193p and Pex11p or between Pex25p and Pex11p, suggesting that Pex11p might act in a pathway independent from that of Yor193p and Pex25p, which might act together in the same pathway. Pex11p has been proposed to initiate peroxisome proliferation in its monomeric form and to terminate peroxisome division when it forms homodimers (Marshall *et al*., 1996). Given that Yor193p and Pex25p are similar to Pex11p in amino acid sequence and in their roles in peroxisome division, Yor193p and Pex25p might act in a manner similar to that of Pex11p in controlling divisional events. Because Pex25p seems to be the least efficient effector of peroxisome division, it is possible that interaction between Yor193p and Pex25p could act as an additional molecular switch to initiate peroxisome division in this second pathway of divisional control. Interestingly, a third PEX11 gene, PEX11λ, has recently been reported from mammals (Li *et al*., 2002; Tanaka *et al*., 2003). Like PEX11β, PEX11λ is constitutively expressed. PEX11λ differs from PEX11α and PEX11β in that its overexpression does not promote peroxisome proliferation. How exactly Pex11α, β and γ interplay in peroxisome proliferation in mammals remains unknown.

Pex11p has been proposed to act in transporting medium-chain fatty acids across the peroxisomal membrane in *S. cerevisiae* and to control peroxisome proliferation indirectly through the generation of a signaling molecule resulting from medium-chain fatty acid oxidation (van Roermund *et al*., 2000). However, this indirect control of peroxisome proliferation by Pex11p has recently been challenged by Li and Gould (2002) who showed that Pex11 proteins could promote peroxisome division in both yeast and mammalian cells in the absence of peroxisomal metabolic activity. They concluded that Pex11 proteins act directly in peroxisome division and that the block of medium-chain fatty acid oxidation observed by van Roermund and colleagues in *S. cerevisiae pex11*Δ cells was the indirect consequence of altered peroxisomal membrane structure or dynamics. Therefore, whether Pex11 proteins, including their relations Yor193p and Pex25p, control peroxisome proliferation directly, or indirectly by modulating peroxisomal metabolism, remains a debated question.

In closing, the maintenance of the size and number of peroxisomes is a tightly controlled process involving separation and division steps. Because of its role in maintaining the size and numbers of peroxisomes, we suggest renaming Yor193p as Pex27p and its encoding gene as *PEX27*. Our results point to Pex11p as the primary regulator of peroxisome division in *S. cerevisiae*, whereas Pex27p and Pex25p act as secondary regulators of this process and have some role in peroxisome separation.

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