Pex3p Initiates the Formation of a Preperoxisomal Compartment from a Subdomain of the Endoplasmic Reticulum in *Saccharomyces cerevisiae*<sup>‡</sup>

Received for publication, June 7, 2005, and in revised form, August 5, 2005 Published, JBC Papers in Press, August 8, 2005, DOI 10.1074/jbc.M506208200

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Peroxisomes are dynamic organelles that often proliferate in response to compounds that they metabolize. Peroxisomes can proliferate by two apparent mechanisms, division of preexisting peroxisomes and de novo synthesis of peroxisomes. Evidence for de novo peroxisome synthesis comes from studies of cells lacking the peroxisomal integral membrane peroxin Pex3p. These cells lack peroxisomes, but peroxisomes can assemble upon reintroduction of Pex3p. The source of these peroxisomes has been the subject of debate. Here, we show that the amino-terminal 46 amino acids of Pex3p of *Saccharomyces cerevisiae* target to a subdomain of the endoplasmic reticulum and initiate the formation of a preperoxisomal compartment for de novo peroxisome synthesis. In *in vivo* video microscopy showed that this preperoxisomal compartment can import both peroxisomal matrix and membrane proteins leading to the formation of bona fide peroxisomes through the continued activity of full-length Pex3p. Peroxisome formation from the preperoxisomal compartment depends on the activity of the genes *PEX14* and *PEX19*, which are required for the targeting of peroxisomal matrix and membrane proteins, respectively. Our findings support a direct role for the endoplasmic reticulum in de novo peroxisome formation.

A characteristic of eukaryotic cells is the presence of seemingly distinct subcellular compartments or organelles possessing specific sets of proteins required for specialized cellular functions. However, organelles do not exist in isolation, and interorganellar communication through the movement and exchange of different molecules is required for normal cell function. This interdependence of organelles extends beyond their biochemical and metabolic roles and necessitates that their biogenesis and turnover also be coordinated.

The peroxisome has long been considered an autonomous organelle that proliferates by the growth and division of preexisting peroxisomes (1) and is inherited as a functional organelle at cell division. But what of the concept of de novo peroxisome biogenesis? From an evolutionary point of view, peroxisome proliferation and inheritance could have evolved as a response to a slow and perhaps unreliable mechanism of de novo peroxisome biogenesis. However, de novo peroxisome biogenesis, when combined with peroxisome growth, division, and inheritance, would provide the cell with a fail-safe system for peroxisome maintenance and ultimately for its survival.

Evidence implicating the endoplasmic reticulum (ER) in peroxisome biogenesis has accumulated in recent years (reviewed in Refs. 2–4). The amino-terminal 16 amino acids of the peroxisomal integral membrane protein Pex3p of *Hansenula polymorpha* were shown to be sufficient to target a reporter protein to the ER (5), whereas treatment of cells of this yeast with brefeldin A led to the accumulation of newly synthesized peroxisomal membrane and matrix proteins at the ER (6). In the yeast *Yarrowia lipolytica*, the peroxisomal membrane proteins Pex2p and Pex16p were shown to traffic through the ER and to acquire core N-linked glycosylation (7). Findings supporting de novo peroxisome biogenesis in close association with the ER were obtained in cells of *Y. lipolytica* temperature-sensitive for Pex3p function (8), and studies in the plant *Arabidopsis* showed that peroxisomal ascorbate peroxidase localized to a subdomain of rough ER that could serve as a compartment for posttranslational sorting to peroxisomes (9). In mouse dendritic cells, the peroxisomal membrane proteins Pex13p and PMP70 were found in subdomains of the ER that extended to a peroxisomal reticulum from which mature peroxisome arose (10).

Little is known about the very early events of peroxisome biogenesis, particularly the formation of the peroxisome membrane. Only Pex3p, Pex16p, and Pex19p have been shown to have specific roles in biogenesis of the peroxisome membrane. Human cells lacking any of these peroxins contain neither peroxisomes nor peroxisome remnants (11–14), whereas cells of *Saccharomyces cerevisiae* deleted for either *PEX3* or *PEX19* appear to lack any type of identifiable peroxisomal structure (15, 16). Functional peroxisomes that were considered to form by de novo peroxisome synthesis were observed upon reintroduction of the *PEX3*, *PEX16*, and *PEX19* genes into their respective mutant cells (11, 12, 14, 17, 18); however, the ultimate source of these newly made peroxisomes remains undefined.

Here, we report the results of studies linking the ER to de novo peroxisome formation in *S. cerevisiae* and show using *in vivo* video microscopy that the amino-terminal 46 amino acids of the peroxin Pex3p initiate the formation of a peroxisomal precursor from the ER membrane from which bona fide peroxisomes can form.

### EXPERIMENTAL PROCEDURES

All experiments were repeated a minimum of three times. The figures present representative images of the individual experiments.

**Yeast Strains, Culture Conditions, and Plasmids**—The *S. cerevisiae* strains used in this study are listed in TABLE ONE. Strains were cul-
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**TABLE ONE**

*S. cerevisiae* strains used in this study

| Strain | Genotype | Ref. |
|--------|----------|------|
| BY4741 | MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0 | 38 |
| BY4742 | MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0 | 38 |
| pex3Δ | MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, pex3:KanMX4 | 38 |
| pex19Δ | MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, pex19:KanMX4 | 38 |
| 46aa-GFP | MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, pex3:46aa-GFP (HIS5) | This study |
| PEX3-GFP | MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, pex3:PEX3-GFP (HIS5) | This study |
| GALPex3/POT1-mRFP | MATa, his3Δ1, leu2Δ0, lys2Δ0, met15Δ0, ura3Δ0, pex3:GALPex3 (KanMX6), pot1::POT1-mRFP (HIS5) | This study |
| GALPex3/FOX2-mRFP-SKL | MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, pex3:GALPex3 (KanMX6), fox2::FOX2-mRFP-SKL (HIS5) | This study |
| GALPex3-mRFP | MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, pex3:GALPex3 (KanMX6), pex3::PEX3-mRFP (HIS5) | This study |
| 46aa-GFP/KAR2-mRFP-HDEL | MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, pex3:46aa-GFP (HIS5), kar2::KAR2-mRFP-HDEL (HIS5) | This study |
| BS9P | MATa/MaTα, his3Δ1/hi3Δ1, leu2Δ0/leu2Δ0, met15Δ0/met15Δ0, +/+ lys2Δ0, ura3Δ0/ura3Δ0, pex3/46aa-GFP (HIS5)/pex3::GALPex3 (KanMX6), pot1::POT1-mRFP (HIS5)/+ | This study |
| BS23F | MATa/MaTα, his3Δ1/hi3Δ1, leu2Δ0/leu2Δ0, met15Δ0/+ , +/+ lys2Δ0, ura3Δ0/+ , pex3/pex3:46aa-GFP (HIS5)/pex3::GALPex3 (KanMX6), fox2::FOX2-mRFP-SKL (HIS5)/+ | This study |
| B5P | MATa/MaTα, his3Δ1/hi3Δ1, leu2Δ0/leu2Δ0, met15Δ0/+ , +/+ lys2Δ0, ura3Δ0/+ , pex3/pex3:46aa-GFP (HIS5)/pex3::GALPex3 (KanMX6) | This study |
| B59P-pex14Δ | MATa/MaTα, his3Δ1/hi3Δ1, leu2Δ0/leu2Δ0, met15Δ0/met15Δ0, +/+ lys2Δ0, ura3Δ0/ura3Δ0, pex14::URA3/pex14::URA3, pex3/46aa-GFP (HIS5)/pex3::GALPex3 (KanMX6), pot1::POT1-mRFP (HIS5)/+ | This study |
| B59P-pex19Δ | MATa/MaTα, his3Δ1/hi3Δ1, leu2Δ0/leu2Δ0, met15Δ0/met15Δ0, +/+ lys2Δ0, ura3Δ0/ura3Δ0, pex19::URA3/pex19::URA3, pex3/46aa-GFP (HIS5)/pex3::GALPex3 (KanMX6), pot1::POT1-mRFP (HIS5)/+ | This study |
| B59P-pex19Δ | MATa/MaTα, his3Δ1/hi3Δ1, leu2Δ0/leu2Δ0, met15Δ0/met15Δ0, +/+ lys2Δ0, ura3Δ0/ura3Δ0, pex19::URA3/pex19::URA3, pex3/46aa-GFP (HIS5)/pex3::GALPex3 (KanMX6) | This study |
| B59P-pex19Δ | MATa/MaTα, his3Δ1/hi3Δ1, leu2Δ0/leu2Δ0, met15Δ0/met15Δ0, +/+ lys2Δ0, ura3Δ0/ura3Δ0, pex19::URA3/pex19::URA3, pex3/46aa-GFP (HIS5)/pex3::GALPex3-mRFP (HIS5) | This study |

Plasmids pFA6a-kanMX6-PGAL1 (19), pRS315 (20), and pRS406 (21) have been described previously. pmRFP-SKL was constructed by replacing the gene for red fluorescent protein in the plasmid pDsRed-PTS1 (22) with the gene encoding monomeric red fluorescent protein (mRFP) (23). pGFP+/-HIS5 and pmRFP-HIS5 were constructed by replacing the gene for GFP in pGFP/HIS5 (24) by the genes encoding GFP+ (25) and mRFP, respectively. A DNA fragment containing 503 bp of sequence downstream of the PEX3 gene was inserted between the BamHI/Xhol sites of pRS315 to make the plasmid pRS315-T. The plasmids p20aa-GFP, p46aa-GFP, and pfull-length-GFP were constructed by inserting DNA fragments containing 497 bp of sequence upstream of the PEX3 gene and sequence encoding the amino-terminal 20 amino acids or the amino-terminal 46 amino acids of Pex3p or full-length Pex3p, respectively, between the XbaI/BamHI sites of pRS315-T followed by insertion of the sequence encoding GFP+ into the BamHI site.

**Integrative Transformation of Yeast**—PCR-based integrative transformation of yeast was used to genomically tag genes with the sequence encoding GFP+ and to introduce the GAL1 promoter upstream of the PEX3 gene by homologous recombination (24).

**Microscopy**—Strains synthesizing GFP+ and/or mRFP chimeras were grown to mid-log phase in synthetic minimal medium and then incubated in YPPO medium for 8 h or SCIM for 16 h. For Raffinose/galactose induction, diploid cells grown overnight in YPR medium were incubated in RIM for 16 h and then transferred to GIM. Images were captured on a LSM510 META (Carl Zeiss) laser scanning microscope or on an Olympus BX50 microscope equipped with a digital fluorescence camera (Spot Diagnostic Instruments). Cells were processed for immunofluorescence microscopy (26) and electron microscopy (27).

**Four-dimensional in Vivo Video Microscopy**—Cells grown in YPR medium and then incubated in RIM for 16 h were prepared for four-dimensional in vivo video microscopy by placing 1–2 μL of culture on a slide with a thin agarose pad containing 2% galactose, which was covered with a coverslip and sealed with petroleum jelly (28). Cells were incubated at room temperature for image capture. Images were captured using a modified LSM 510 META confocal microscope equipped with a ×63 1.4 normal aperture Plan-Apo objective (Carl Zeiss) (29). A piezoelectric actuator was used to drive continuous objective movement, allowing for rapid collection of z-stacks. A side of each pixel representative 0.085 μm of sample. Stacks of eight optical sections spaced 0.45 μm apart were captured every 60 s. GFP was excited using a 488-nm laser, and its emission was collected using a 505–530-nm bandpass filter. mRFP was excited using a 543-nm laser, and its emission was collected using a 600-nm long-pass filter. Images were filtered three times using a 3 × 3 hybrid median filter to reduce shot noise. Fluorescence images from each stack were projected using an average intensity algorithm that involved multiplication of each pixel value by an appro-
Appropriate enhancement factor for better contrast. Correction for exponential photobleaching of GFP and mRFP was performed by exponentially increasing the enhancement factor with each projection. The transmitted light images from each stack were projected using a maximum intensity algorithm. These operations were performed using NIH Image (rsb.info.nih.gov/nih-image/). Adobe Photoshop was used to merge fluorescent and transmitted light projections.

**Antibodies**—Antibodies to amino acids 43–440 of Pex3p and to GFP were raised in rabbit and guinea pig. Antibodies to the carboxyl-terminal SKL tripeptide (30) and to peroxisomal thiolase (31) have been described previously. Rabbit antibodies to S. cerevisiae glucose-6-phosphate dehydrogenase were obtained from Sigma. Horseradish peroxidase-conjugated donkey anti-rabbit IgG and horseradish peroxidase-conjugated goat anti-guinea pig IgG secondary antibodies (Amersham Biosciences) 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) were used to detect primary antibodies in immunofluorescence microscopy.

**Analytical Procedures**—Subcellular fractionation was performed as described previously (22). Whole cell lysates were prepared as described previously (27). Antigen-antibody complexes in immunoblots were detected by enhanced chemiluminescence (Amersham Biosciences).

**RESULTS**

**Truncated Pex3p Proteins Reveal Peroxisomal Localization Information for Pex3p and the Presence of an Unknown Compartment**—Pex3p and Pex19p act early in the biogenesis of peroxisomes in S. cerevisiae as cells lacking either peroxin do not contain peroxisomes, and peroxisomes can be observed to form upon their reintroduction. Pex3p is reported to be the docking factor for Pex19p on the peroxisomal membrane (32). This observation places the function of Pex3p in peroxisome biogenesis ahead of that of Pex19p; therefore, Pex3p serves as the best candidate protein with which to study the early events of peroxisome biogenesis.

Genes encoding GFP fused to the amino-terminal 20 (20aa-GFP) or 46 (46aa-GFP) amino acids of Pex3p or to full-length Pex3p (Pex3p-GFP) were expressed under the control of the native PEX3 promoter from plasmid in the parental haploid strain BY4741 and in the peroxisome-deficient strains pex3Δ and pex19Δ. pmRFP-PTS1 was cotransformed into the various strains to fluorescently label peroxisomes, and
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cells were grown in oleic acid medium and analyzed by confocal microscopy (Fig. 1A). Pex3p-GFP was able to target to peroxisomes in BY4741 and pex3Δ cells, as shown by the colocalization of GFP and mRFP signals in punctate structures. However, in pex19Δ cells, Pex3p-GFP was targeted to punctate structures that did not fluorescently label with mRFP-PTS1 (which labeled the cytosol) and therefore do not correspond to peroxisomes, confirming that the formation of peroxisomes requires a copy of PEX19. The 20aa-GFP chimera localized to the cytosol of all strains despite the fact that BY4741 cells contain peroxisomes. pex3Δ cells expressing 20aa-GFP were unable to form peroxisomes, suggesting that the information for the formation of peroxisomes is not encompassed by the first 20 amino acids of Pex3p. 46aa-GFP was targeted to peroxisomes in BY4741 cells. Because 20aa-GFP was unable to target to peroxisomes, the peroxisome targeting signal (PTS) of Pex3p must extend to between amino acids 21 and 46. Interestingly, in pex3Δ and pex19Δ cells, 46aa-GFP localized to an unknown compartment represented by one or two small punctate fluorescent structures. These structures were not peroxisomes, as they did not label with mRFP-PTS1, which mislocalized to the cytosol. The capacity of 46aa-GFP to be targeted to peroxisomes in BY4741 cells that contain peroxisomes and to an unknown compartment in pex3Δ and pex19Δ cells that lack peroxisomes suggested that this unknown compartment might serve as a preperoxisomal compartment from which peroxisomes could form upon provision of cells with full-length Pex3p. Targeting of 46aa-GFP to the unknown compartment is independent of Pex19p, consistent with a previous report that Pex19p is not required to target Pex3p to peroxisomes (32).

To avoid possible artifacts of gene overexpression from multicopy plasmids, genetically encoded GFP chimeras of Pex3p (gPex3p-GFP) and the amino-terminal 46 amino acids of Pex3p (g46aa-GFP) were constructed. As observed with construct expression from plasmid (Fig. 1A), epifluorescence analysis of oleic acid-incubated cells showed that gPex3p-GFP localized to punctate structures with the characteristics of peroxisomes, whereas g46aa-GFP localized to an unknown compartment that presented usually as one or two fluorescent dots (Fig. 1B). Immunofluorescence analysis of oleic acid-incubated cells with antibodies to the carboxyl-terminal PTS1 tripeptide Ser-Lys-Leu (SKL) or to the PTS2-containing enzyme Pot1p (thiolase) showed that cells expressing gPex3p-GFP contained peroxisomes having both Pot1p- and PTS1-containing proteins, as observed for parental BY4741 cells (Fig. 1C). In contrast, cells expressing g46aa-GFP showed a cytosolic location for both Pot1p- and PTS1-containing proteins, as in pex3Δ cells, consistent with the absence of peroxisomes in both cell types (Fig. 1C). The functionality of the GFP chimeras was determined by growing cells on agar medium containing oleic acid as the sole carbon source, the metabolism of which requires functional peroxisomes. Cells expressing Pex3p-GFP grew at a rate similar to that of BY4741 cells (Fig. 1D), suggesting that gPex3p-GFP functions like wild-type Pex3p. As expected, pex3Δ cells failed to grow. Cells expressing g46aa-GFP grew poorly or not at all, indicating that peroxisomal function is compromised in these cells. In electron micrographs, peroxisomes of BY4741 cells incubated in oleic acid-containing medium appeared as typical round vesicular structures, 0.1–0.5 μm in diameter, surrounded by a single unit membrane and containing a homogenous granular matrix (Fig. 1E) (22, 26, 27). In contrast, pex3Δ cells and cells expressing g46aa-GFP lacked identifiable peroxisomes.

The Amino Terminus of Pex3p Targets a Subdomain of the ER—Because cells expressing g46aa-GFP do not contain peroxisomes (Fig. 1C), we attempted to define the subcellular compartment containing the chimera by performing colocalization analyses of g46aa-GFP with known organellar markers. g46aa-GFP did not colocalize with mitochondria marked with MitoTracker dye but showed an almost absolute colocalization with a genomically encoded fluorescent chimera (gKar2p-mRFP-HDEL) of the ER-resident chaperone, Kar2p (Fig. 2A). In 100 cells, 81.5% of g46aa-GFP-containing structures colocalized with gKar2p-mRFP-HDEL (TABLE TWO). Subcellular fractionation also supported localization of g46aa-GFP to the ER compartment (Fig. 2B). Pex3p in BY4741 cells localized mainly to the 20Kg fraction enriched for heavy organelles, including peroxisomes. Kar2p also localized preferentially to the 20Kg fraction, but a substantial fraction of Kar2p was also detected in the 20KgS fraction enriched for cytosol and lighter organelles. g46aa-GFP localized almost exclusively to the 20KgS fraction. Upon ultracentrifugation of the 20KgS fraction, g46aa-GFP cofractionated to both the 250KgS and 250KgP fractions in a manner almost identical to that of Kar2p, consistent with a colocalization of g46aa-GFP and that portion of Kar2p initially found in the 20KgS fraction. Together these results suggest that the previously unknown compartment to which g46aa-GFP targets is a subdomain of the ER.

Peroxisomes Form from the Compartment Targeted by the Amino Terminus of Pex3p—Might the g46aa-GFP-containing compartment be a preperoxisomal compartment? To investigate the dynamics of this compartment upon synthesis of full-length Pex3p, haploid cells expressing g46aa-GFP were mated to haploid cells expressing Pex3p under the control of the GAL1 promoter. Cells were grown in raffinose/oleic acid medium (RIM) and the diploid cells were analyzed by epifluorescence microscopy at various times after transfer to GIM (Fig. 3A). At 0 h, both matrix enzymes were localized to the cytosol, and no signal for Pex3p-mRFP was observed. With increased synthesis of Pex3p-mRFP at later times, Pex3p-mRFP appeared in and colocalized with the punctate structures labeled by g46aa-GFP. Upon induction of Pex3p, both Pot1p-mRFP and Fox2p-mRFP-SKL also targeted to the g46aa-GFP-labeled

FIGURE 2. g46aa-GFP targets to a subdomain of the ER. A, confocal microscopy showing a single z-plane of YPBO-incubated cells expressing g46aa-GFP and gKar2p-mRFP-HDEL and YPBO-incubated cells expressing g46aa-GFP and treated with MitoTracker to visualize mitochondria. B, immunoblot analysis of 20KgS, 20KgP, 250KgS, and 250KgP subcellular fractions from 46aa-GFP and BY4741 cells incubated in YPBO with antibodies to GFP, Kar2p, and Pex3p. Equivalent portions of each fraction were analyzed.
structures. Electron micrographs of the diploid strain B59P showed no recognizable peroxisomal structures at 0 h but showed characteristic peroxisomes at 6 h after transfer to GIM (Fig. 3B). It is noteworthy that cells showed peroxisome clustering after being incubated in GIM for more than 4 h (Fig. 3, A and B), possibly because of high levels of synthesis of Pex3p (Fig. 3C). In contrast, during incubation in GIM, the levels of g46aa-GFP decreased over time, suggesting that galactose inhibits the normal induction pattern observed for Pex3p in oleic acid-containing medium.

Four-dimensional in vivo video microscopy has proven a valuable tool with which to study the dynamics of cellular processes. It has recently been used to observe the de novo formation of transitional ER sites and Golgi structures (33). We used four-dimensional in vivo video microscopy to visualize the dynamics of the g46aa-GFP compartment and the formation of peroxisomes. Diploid B5P3 and B59P cells were grown in RIM, transferred to GIM for 1.5 h, and then spotted onto an agarose pad containing oleic acid and a small quantity of glucose (SCIM). Cells grown in RIM for 16 h were transferred to GIM for 1.5 h and placed onto a slide covered with a thin agarose pad containing SCIM and galactose. Cells were visualized at room temperature on a LSM 510 META confocal microscope specially modified for four-dimensional in vivo video microscopy (see “Experimental Procedures”). Representative frames from supplemental videos show the import of gPex3p-mRFP and gPot1p-mRFP into the g46aa-GFP-labeled compartment. Numbers indicate the time in minutes. A, continuous targeting of gPex3p-mRFP into the g46aa-GFP-labeled structures in B5P3 cells (see also supplemental video 1). B, progressive import of gPot1p-mRFP from the cytosol into g46aa-GFP-labeled structures in B59P cells (see also supplemental video 2). Partitioning of peroxisomes from mother cells to buds occurs between 180 and 269 min. Selected frames of both color channels were individually displayed as black and white images.

**TABLE TWO**

| Strains                     | Number of punctate GFP structures in 100 cells | Number of punctate GFP structures colocalizing with gKar2p-mRFP-HDEL |
|-----------------------------|-----------------------------------------------|---------------------------------------------------------------------|
| g46aa-GFP/KAR2-mRFP-HDEL    | 157                                           | 128                                                                 |
| KAR2-mRFP-HDEL              | 0                                             | NA*                                                                 |
| g46aa-GFP                   | 161                                           | NA                                                                  |
| 20aa-GFP                    | 0                                             | NA                                                                  |
| 20aa-GFP/KAR2-mRFP-HDEL     | 0                                             | 0                                                                   |

* NA, not applicable.
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Figure 5: Formation of peroxisomes, but not targeting of Pex3p, depends on PEX14 and PEX19. B59P and B5P3 cells deleted for PEX14 and PEX19 were shifted from RIM containing raffinose and oleic acid to GIM containing galactose and oleic acid. Cells were removed from GIM at the times indicated and analyzed by epifluorescence microscopy. Bar, 1 μm.

g46aa-GFP construct might delay the exit of the peroxisomal precursor from the ER compartment, as it lacks those sequences of Pex3p downstream of the first amino-terminal amino acids responsible for this process. As we have observed, expression of full-length Pex3p together with g46aa-GFP led to the formation of bona fide peroxisomes capable of peroxisomal matrix and membrane protein import from the cytosol (Figs. 3A and 4), consistent with information for the exit of the peroxisomal precursor from the ER being provided by sequences of the Pex3p carboxyl-terminal to its first 46 amino acids. It should be noted that some mother cells expressing g46aa-GFP and full-length Pex3p appeared to be devoid of peroxisomes (Fig. 4). However, peroxisomes capable of importing matrix (gPot1p-mRFP) and membrane (gPex3p-mRFP) proteins were observed in mother cells after prolonged periods of incubation. We did not observe the reappearance of typical g46aa-GFP-labeled structures in these cells, possibly because of rapid photobleaching of newly synthesized GFP.

Peroxisome Formation from the Preperoxisomal Compartment Requires Pex14p and Pex19p—Both Pex14p and Pex19p are required for peroxisome assembly. Pex14p is the point of convergence of the PTS1 and PTS2 matrix protein import pathways (34). Pex19p is required for stability in and/or targeting to the peroxisomal membrane for most peroxisomal membrane proteins (14, 35). We therefore investigated what roles Pex14p and Pex19p might have in regulating the dynamics of the g46aa-GFP-labeled compartment. Cells lacking Pex14p or Pex19p were grown in RIM and then transferred to GIM, and aliquots of cells were taken at various times after transfer. The fluorescence signals for g46aa-GFP, Pot1p-mRFP, and Pex3p-mRFP were analyzed (Fig. 5). Cells deleted for either PEX14 or PEX19 showed colocalization of g46aa-GFP with Pex3p-mRFP when shifted to GIM, and the synthesis of Pex3p-mRFP was turned on. However, Pot1p-mRFP remained mostly cytosolic and was not targeted to g46aa-GFP-labeled structures. Therefore, both PEX14 and PEX19 are required for the development of g46aa-GFP-labeled structures into peroxisomes.

DISCUSSION

This study shows that the amino terminus of Pex3p targets to peroxisomes in wild-type cells and a subdomain of the ER in cells lacking peroxisomes. This subdomain of the ER can develop into functional peroxisomes through the activity of full-length Pex3p.

Immunoelectron microscopy of mouse dendritic cells has shown that the peroxisomal membrane protein Pex13p can be found in a specialized ER subdomain (10). Three-dimensional image reconstruction demonstrated continuity between this specialized ER subdomain and a reticular structure resembling peroxisomes. These results suggest a peroxisome maturation pathway initiating at the ER. However, a peroxisomal reticulum has not been observed in yeasts. We were unable to observe any unique membranous structure in electron micrographs that might correspond to the punctate structure targeted by g46aa-GFP. This is not surprising given that the preperoxisomal vesicles of Y. lipolytica have a rather routine appearance that does not distinguish them from the overall population of vesicles in the cell (36).

To support a model for peroxisome maturation that initiates at the level of the ER, it is important to show the development of peroxisomes in relation to the ER in terms of the import of both peroxisomal membrane and matrix proteins. Using four-dimensional in vivo video microscopy, we showed the targeting of the peroxisomal membrane chimeric protein Pex3p-mRFP to punctate structures (Fig. 3A) that exhibited both the morphological (Fig. 2A) and biochemical (Fig. 2B) characteristics of a subdomain of the ER. The formation of this compartment was initiated by the expression of g46aa-GFP (Fig. 2A), and this compartment was also able to import fluorescently labeled derivatives of the PTS1-containing matrix protein Fox2p (gFox2p-mRFP-SKL) and the PTS2-containing matrix protein Pot1p (gPot1p-mRFP) (Fig. 3A).

How this preperoxisomal compartment actually dissociates itself from the ER remains unknown. The targeting of the membrane proteins Pex2p, Pex3p, and Pex16p to peroxisomes was unaffected in mammalian cells blocked in COPI- or COPII-mediated vesicular transport (17, 37). However, experiments in H. polymorpha showed that a subset of peroxisomal proteins was trapped in the ER in cells treated with brefeldin A (6). A possible role for COPI and COPII in peroxisome formation has yet to be investigated in S. cerevisiae. How g46aa-GFP reaches the ER is also unknown. Pex3p might have intrinsic properties that direct it to the ER, or other proteins might aid in delivering Pex3p to the ER. Inactivation of the ER translocation machinery components Sec61p and Ssh1p did not have an effect on peroxisome biogenesis (18). This result has been taken by some researchers as proof that the ER was not involved in peroxisome biogenesis. However, proteins could enter the ER via some undefined mechanism independent of Sec61p or Ssh1p. Future experiments aimed at reconstituting in vitro the import of Pex3p into the ER should clarify this process. Our findings demonstrating a requirement for Pex14p and Pex19p in the formation of peroxisomes from the g46aa-GFP-labeled preperoxisomal compartment that are also capable of matrix protein import from the cytosol (Fig. 5) are consistent with a scenario in which Pex19p docks to Pex3p to facilitate the import of other peroxisomal membrane proteins such as Pex14p (32).

In conclusion, we show that the peroxisomal integral membrane protein Pex3p traffics through the ER and participates in the formation of...
preperoxisomal vesicles from this endomembrane system. Through the continued activity of Pex3p, these preperoxisomal vesicles can develop into bona fide peroxisomes via the import of peroxisomal matrix proteins. Our findings demonstrate a direct role for the ER in the de novo formation of peroxisomes.

Acknowledgments—We thank Elena Savidov, Dwayne Weber, Hanna Kroliczak, and Richard Poirier for technical help and Honey Chan for assistance with electron microscopy.

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