Selective degradation of peroxisomes (macropexophagy) in Hansenula polymorpha involves the sequestration of individual organelles to be degraded by membranes prior to the fusion of this compartment with the vacuole and subsequent degradation of the whole organelle by vacuolar hydrolases. Here we show that Pex3p, a peroxisomal membrane protein essential for peroxisome biogenesis, escapes this autophagic process. Upon induction of macroautophagy, Pex3p is removed from the organelle tagged for degradation prior to its sequestration. Our data indicate that Pex3p degradation is essential to allow the initiation of the organelar degradation process. Also, in a specific peroxisome degradation-deficient (pdd) mutant in which sequestration still occurs but the vacuolar fusion event is disturbed, the turnover of Pex3p is still observed. Taken together, our data suggest that degradation of Pex3p is part of the initial degradation machinery of individual peroxisomes.

Peroxisomes are important organelles that play a role in various metabolic processes in eukaryotes. In fungi, they are predominantly involved in the oxidative metabolism of the carbon and/or nitrogen source used for growth. Characteristically, during cultivation on compounds that require the function of peroxisomal enzymes, the organelles rapidly develop and contain the key enzymes involved in the metabolism of the specific growth substrate. The opposite also occurs. When cells grown at peroxisome-inducing conditions are placed in fresh media in which these functions are no longer required for growth, the organelles are rapidly and selectively degraded by an autophagic process, also termed pexophagy (1–2).

In Hansenula polymorpha, selective degradation of peroxisomes is induced when methanol-grown cells are shifted to fresh glucose- or ethanol-containing media. This process involves three morphologically distinct steps, namely (i) sequestration of the organelle to be degraded from the cytosol by various membranous layers, (ii) the fusion of the outer membranous layer of the sequestered compartment with the vacuolar membrane followed by (iii) the degradation of the organelar components by vacuolar hydrolases (3). This process has been designated macropexophagy.

In the related species Pichia pastoris, a similar process takes place after a shift of cells from methanol to ethanol. However, when glucose is used to induce pexophagy, an alternative degradation pathway is initiated termed micropexophagy. The hallmark of this process is that clusters of peroxisomes are engulfed by the vacuole (4).

Recently, we observed that the mechanisms of peroxisome biogenesis and selective degradation in H. polymorpha use factors in common. We demonstrated that Pex14p, a crucial component in matrix protein import, is also essential for selective peroxisome degradation in H. polymorpha (5, 6). Although the molecular mechanisms of the dual function of Pex14p in the two oppositely directed processes are still unresolved, we showed that the information that controls degradation resides in the N terminus of the protein (6).

Here, we show that a second peroxin Pex3p, which is essential for peroxisome biogenesis and maintenance (7), also plays a role in macropexophagy in H. polymorpha. At present, a consensus exists that upon induction of macropexophagy in yeast, the whole organelle is degraded by vacuolar hydrolases. In this paper, we demonstrate that Pex3p escapes this process and is degraded prior to uptake of the organelle by the vacuole. We have interpreted our data in that degradation of Pex3p is an essential early step in selective degradation of individual peroxisomes by macropexophagy.

MATERIALS AND METHODS

Organisms, Cultivation Media, and Conditions—The H. polymorpha strains used in this study are described in Table I. H. polymorpha cells were grown in batch cultures at 37 °C on (a) selective minimal medium containing 0.67% (w/v) yeast nitrogen base without amino acids (Difco) supplemented with 1% (w/v) glucose, (b) rich medium containing 1% (w/v) yeast extract, 1% (w/v) peptone, and 1% (w/v) glucose, or (c) mineral medium (8) supplemented with 0.5% (w/v) glucose, 0.5% (v/v) methanol, or a mixture of 0.1% (v/v) glycerol and 0.5% (v/v) methanol as carbon source together with 0.25% (w/v) ammonium sulfate as nitrogen source. When required, leucine (30 µg/ml) was added. During proteasome inhibition experiments, the proteasome inhibitor MG-132 was added to the culture to a final concentration of 50 µM (9).

For analysis of pexophagy, cells were extensively pre-cultivated on minimal medium containing glucose and shifted to minimal medium containing methanol to induce pexophagy proliferation. 0.5% glucose was added to induce selective pexophagy degradation. Escherichia coli DH5α (10) was used for plasmid amplification and was grown on LB medium supplemented with the appropriate antibiotics.

Molecular Techniques—Standard recombinant DNA techniques (10) and transformation of H. polymorpha was performed as described previously (11). Restriction enzymes and biochemicals were obtained from Roche Molecular Biochemicals and used as detailed by the manufacturer.

Construction of the PEX3.GFP Strain—An H. polymorpha wild type strain in which the expression of the hybrid gene PEX3.GFP is regulated by the endogenous PEX3 promoter (P_{PEX3}) was constructed as...
 Degradation of Pex3p in pdd1 Mutant Strains—To further analyze this phenomenon, we analyzed cells that lacked the membrane protein Pex14p. As shown before, Pex14p is essential for peroxisome biogenesis, and normal peroxisomes are absent in PEX14 depletion strains (pex14) (13). However, in H. polymorpha pex14 cells, peroxisome development can be restored by the overproduction of the PTS1 receptor, Pex5p (17). Also, because of the absence of Pex14p, these organelles are no longer susceptible to selective degradation (5). After a shift of methanol-induced H. polymorpha pex14 cells that overproduce Pex5p (pex14::P_{AOX}PEX5^{666}) to glucose-containing medium. Pex3p is again rapidly degraded, whereas Pex10p, an integral component of the peroxisomal membrane remained virtually unaffected (Fig. 1B). Essentially, similar results were obtained using cells producing a truncated form of Pex14p of which the first 64 amino acids are deleted (PEX14–ΔN64), and for that reason, they cannot degrade peroxisomes (Fig. 1C). Therefore, these results are in line with the assumption that the degradation pathway of Pex3p differs from that of other peroxisomal proteins upon the induction of selective peroxisome degradation in H. polymorpha.

### Selective Peroxisome Degradation in WT Cells at Different Growth Stages—In previous experiments on WT H. polymorpha cells, the phenomenon of an alternative rapid Pex3p degradation pathway was less evident (14). We reasoned that this might be related to microbody numbers, and thus, to the growth stage of the cells. It is well documented that the cells of H. polymorpha that are in the early exponential growth phase on methanol generally contain a single peroxisome and that the peroxisomal volume fraction may increase over 20-fold during subsequent growth (18). In conventional degradation experiments, cells have been used that were in the late exponential growth phase and, thus, had accumulated numerous peroxisomes. Because peroxisomes in H. polymorpha are degraded individually and sequentially, the relative high numbers of peroxisomes in such cells in fact could mask the rapid degradation of Pex3p from the relatively few organelles that are

## RESULTS

### Degradation of Pex3p in pdd1 Mutant Strains

In general, selective peroxisome degradation in H. polymorpha is monitored by following the level of one of the major matrix enzymes: AO, dihydroxyacetone synthase, or catalase. In a conventional degradation experiment using this organism, the level of AO activity and protein decreases to approximately 10–20% of the original value within the first 4 h after the shift (3, 14, 15). Comparable observations have been made for peroxisomal membrane proteins. However, in the course of our experiments, we noticed that in cells of specific H. polymorpha mutants, which are impaired in selective peroxisome degradation (pdd mutants) (15), the peroxisomal membrane protein Pex3p nevertheless is degraded upon the addition of glucose. An example of this is shown in Fig. 1A. After a shift of methanol-grown pdd1 (15) cells to glucose-containing medium, AO protein is not degraded as expected. However, in the same cells, the level of Pex3p is drastically reduced within a time interval of 90 min. (Fig. 1A).

### Degradation of Pex3p in pdd2 Mutant Strains

In general, selective peroxisome degradation in H. polymorpha is monitored by following the level of one of the major matrix enzymes: AO, dihydroxyacetone synthase, or catalase. In a conventional degradation experiment using this organism, the level of AO activity and protein decreases to approximately 10–20% of the original value within the first 4 h after the shift (3, 14, 15). Comparable observations have been made for peroxisomal membrane proteins. However, in the course of our experiments, we noticed that in cells of specific H. polymorpha mutants, which are impaired in selective peroxisome degradation (pdd mutants) (15), the peroxisomal membrane protein Pex3p nevertheless is degraded upon the addition of glucose. An example of this is shown in Fig. 1A. After a shift of methanol-grown pdd2 (15) cells to glucose-containing medium, AO protein is not degraded as expected. However, in the same cells, the level of Pex3p is drastically reduced within a time interval of 90 min. (Fig. 1A).

### Degradation of Pex3p in pdd2–Pex14 Mutant Strains

In general, selective peroxisome degradation in H. polymorpha is monitored by following the level of one of the major matrix enzymes: AO, dihydroxyacetone synthase, or catalase. In a conventional degradation experiment using this organism, the level of AO activity and protein decreases to approximately 10–20% of the original value within the first 4 h after the shift (3, 14, 15). Comparable observations have been made for peroxisomal membrane proteins. However, in the course of our experiments, we noticed that in cells of specific H. polymorpha mutants, which are impaired in selective peroxisome degradation (pdd mutants) (15), the peroxisomal membrane protein Pex3p nevertheless is degraded upon the addition of glucose. An example of this is shown in Fig. 1A. After a shift of methanol-grown pdd2 (15) cells to glucose-containing medium, AO protein is not degraded as expected. However, in the same cells, the level of Pex3p is drastically reduced within a time interval of 90 min. (Fig. 1A).

### Degradation of Pex3p in pdd1–Pex14 Mutant Strains

In general, selective peroxisome degradation in H. polymorpha is monitored by following the level of one of the major matrix enzymes: AO, dihydroxyacetone synthase, or catalase. In a conventional degradation experiment using this organism, the level of AO activity and protein decreases to approximately 10–20% of the original value within the first 4 h after the shift (3, 14, 15). Comparable observations have been made for peroxisomal membrane proteins. However, in the course of our experiments, we noticed that in cells of specific H. polymorpha mutants, which are impaired in selective peroxisome degradation (pdd mutants) (15), the peroxisomal membrane protein Pex3p nevertheless is degraded upon the addition of glucose. An example of this is shown in Fig. 1A. After a shift of methanol-grown pdd1 (15) cells to glucose-containing medium, AO protein is not degraded as expected. However, in the same cells, the level of Pex3p is drastically reduced within a time interval of 90 min. (Fig. 1A).

### Degradation of Pex3p in pdd2–Pex14 Mutant Strains

In general, selective peroxisome degradation in H. polymorpha is monitored by following the level of one of the major matrix enzymes: AO, dihydroxyacetone synthase, or catalase. In a conventional degradation experiment using this organism, the level of AO activity and protein decreases to approximately 10–20% of the original value within the first 4 h after the shift (3, 14, 15). Comparable observations have been made for peroxisomal membrane proteins. However, in the course of our experiments, we noticed that in cells of specific H. polymorpha mutants, which are impaired in selective peroxisome degradation (pdd mutants) (15), the peroxisomal membrane protein Pex3p nevertheless is degraded upon the addition of glucose. An example of this is shown in Fig. 1A. After a shift of methanol-grown pdd2 (15) cells to glucose-containing medium, AO protein is not degraded as expected. However, in the same cells, the level of Pex3p is drastically reduced within a time interval of 90 min. (Fig. 1A).

### Degradation of Pex3p in pdd1–Pex14 Mutant Strains

In general, selective peroxisome degradation in H. polymorpha is monitored by following the level of one of the major matrix enzymes: AO, dihydroxyacetone synthase, or catalase. In a conventional degradation experiment using this organism, the level of AO activity and protein decreases to approximately 10–20% of the original value within the first 4 h after the shift (3, 14, 15). Comparable observations have been made for peroxisomal membrane proteins. However, in the course of our experiments, we noticed that in cells of specific H. polymorpha mutants, which are impaired in selective peroxisome degradation (pdd mutants) (15), the peroxisomal membrane protein Pex3p nevertheless is degraded upon the addition of glucose. An example of this is shown in Fig. 1A. After a shift of methanol-grown pdd1 (15) cells to glucose-containing medium, AO protein is not degraded as expected. However, in the same cells, the level of Pex3p is drastically reduced within a time interval of 90 min. (Fig. 1A).

### Degradation of Pex3p in pdd1–Pex14 Mutant Strains

In general, selective peroxisome degradation in H. polymorpha is monitored by following the level of one of the major matrix enzymes: AO, dihydroxyacetone synthase, or catalase. In a conventional degradation experiment using this organism, the level of AO activity and protein decreases to approximately 10–20% of the original value within the first 4 h after the shift (3, 14, 15). Comparable observations have been made for peroxisomal membrane proteins. However, in the course of our experiments, we noticed that in cells of specific H. polymorpha mutants, which are impaired in selective peroxisome degradation (pdd mutants) (15), the peroxisomal membrane protein Pex3p nevertheless is degraded upon the addition of glucose. An example of this is shown in Fig. 1A. After a shift of methanol-grown pdd1 (15) cells to glucose-containing medium, AO protein is not degraded as expected. However, in the same cells, the level of Pex3p is drastically reduced within a time interval of 90 min. (Fig. 1A).
The Role of Pex3p in Peroxisome Degradation

Selective peroxisome degradation was induced in methanol-grown cells at the early exponential growth phase (OD₆₆₃ = 0.8) (A) or late exponential growth phase (OD₆₆₃ = 2.0) (B) by the addition of glucose. Samples were taken at regular time intervals after the addition of glucose (t = 0 h). Samples corresponding to equal volumes of the cultures were loaded per lane. Blots were decorated using antibodies against ScPex3p, Pex14p, and Pex10p. In cells from the early exponential growth phase (A), Pex3p levels dropped rapidly compared with AO and Pex14p. This difference is much less clear when glucose was added to methanol-grown cells at the late exponential growth phase (B).

Therefore, we decided to analyze the macropexophagy process in cells that contained only few peroxisomes and compared the data with those obtained in cells from the late exponential growth phase in which they contained numerous peroxisomes.

The Role of Pex3p in Macropexophagy

Electron microscopical analysis confirmed that peroxisomes were not degraded in the presence of MG-132. In these cells, the peroxisome degradation process appeared to be disturbed at an early stage, namely sequestration. As expected, during normal pexophagy in the absence of MG-132, individual peroxisomes were sequestered from the cytosol by several membrane layers (Fig. 4A). Also, in the presence of MG-132, additional membranes were formed. However, they failed to fully and closely envelop the peroxisomes (Fig. 4B).

ScPex3p and Fusion of GFP to HpPex3p Affect Macropexophagy—We next analyzed whether the authentic H. polymorpha Pex3p was required to allow macropexophagy to occur. To this end, we analyzed peroxisome degradation in WT H. polymorpha in relation to the cultivation conditions. Selective peroxisome degradation was induced in methanol-grown cells at the early exponential growth phase (OD₆₆₃ = 0.8) (A) or late exponential growth phase (OD₆₆₃ = 2.0) (B) by the addition of glucose. Samples were taken at regular time intervals after the addition of glucose (t = 0 h). Samples corresponding to equal volumes of the cultures were loaded per lane. Blots were decorated with antibodies against AO, Pex14p, and Pex3p. In cells from the early exponential growth phase (A), Pex3p levels dropped rapidly compared with AO and Pex14p. This difference is much less clear when glucose was added to methanol-grown cells at the late exponential growth phase (B).

Electron microscopical analysis confirmed that peroxisomes were not degraded in the presence of MG-132. In these cells, the peroxisome degradation process appeared to be disturbed at an early stage, namely sequestration. As expected, during normal pexophagy in the absence of MG-132, individual peroxisomes were sequestered from the cytosol by several membrane layers (Fig. 4A). Also, in the presence of MG-132, additional membranes were formed. However, they failed to fully and closely envelop the peroxisomes (Fig. 4B).
The Role of Pex3p in Peroxisome Degradation

FIG. 6. Peroxisome degradation is prevented in *H. polymorpha* pex3::P<sub>PEX3</sub>ScPEX3. Immunocytochemical localization of AO protein in methanol-grown cells 60 min after the addition of glucose. In *H. polymorpha* cells in which the HpPEX3 gene has been replaced by the *S. cerevisiae* PEX3 gene (pex3::P<sub>PEX3</sub>ScPEX3), anti-AO-dependent labeling is only observed on peroxisomes and is invariably absent on vacuoles, confirming that peroxisomes are not degraded in these cells (A). In WT control cells, AO labeling is observed both on peroxisome and vacuole profiles, indicative for the ongoing peroxisome degradation (B). Ultra-thin sections of glutaraldehyde-fixed cells labeled with anti-AO antibodies are shown. The bar represents 0.5 μm. AV, autophagic vacuole; M, mitochondrion; N, nucleus; F, peroxisome.

Pex3p degradation in an available *H. polymorpha* pex3 strain that was functionally complemented by the *Saccharomyces cerevisiae* PEX3 gene under control of the endogenous *H. polymorpha* PEX3 promoter, pex3::P<sub>PEX3</sub>ScPEX3. This strain can normally grow on methanol and displays normal WT properties in peroxisome biogenesis and function (19). However, after a shift of methanol-grown cells of this strain to excess glucose conditions, the level of ScPex3p did not decrease. Moreover, also the level of AO remained unaltered, indicating that peroxisome degradation did not occur (Fig. 5A). Electron microscopical analysis confirmed that the ScPex3p containing peroxisomes were not degraded in these cells (Fig. 6). In WT controls, a significant anti-AO-dependent labeling was found both on peroxisome and vacuole profiles 60 min after the shift of cells to glucose, a phenomenon indicative for vacuolar degradation of peroxisomes. However, in pex3::P<sub>PEX3</sub>ScPEX3, cells shifted to glucose excess conditions and anti-AO labeling was confined to peroxisomes, whereas vacuoles were invariably devoid of anti-AO labeling (Fig. 6).

Fusion of Green Fluorescent Protein (GFP)<sup>1</sup> to the extreme C terminus of *H. polymorpha* Pex3p also strongly interfered with macroperoxophagy. In a conventional degradation experiment, the levels of HpPex3p-GFP and AO decreased much slower (Fig. 5B) compared with Pex3p and AO in WT cells. Apparently, the GFP tag interferes with the rapid degradation of Pex3p and the subsequent turnover of the organelle.

**Rapid Pex3p Degradation Does Not Occur during Normal Growth of Cells on Methanol**—To test whether Pex3p degradation is a rapid constitutive process and also occurs under conditions where peroxisome degradation is not induced, we tested the effect of the proteasome inhibitor MG-132 on Pex3p levels in cultures that were in the exponential growth phase on methanol. As shown in Fig. 7, the addition of MG-132 to such cells did not result in an increase in Pex3p levels relative to controls that lack MG-132. These data indicate that at normal growth conditions, no strong continuous Pex3p turnover occurs. Instead, they strongly support the notion that the decline of Pex3p upon initiation of pexophagy is the result of induced Pex3p degradation rather than a shift in equilibrium of Pex3p synthesis, which is reduced because of the addition of glucose, and continuous degradation.

**DISCUSSION**

In this paper, we provide evidence that upon the induction of selective degradation of peroxisomes (macropexophagy) in *Hansenula polymorpha* a peroxisomal membrane protein, Pex3p, does not follow the normal degradative pathway of other peroxisomal constituents in the vacuole.

Pex3p is one of the first identified peroxisomal membrane proteins and has been observed in various organisms including man (7, 20–23). Recently, a Zellweger syndrome patient belonging to complementation group G was shown to be affected in Pex3p function (24). In *H. polymorpha*, the protein is essential for peroxisome biogenesis and maintenance (7). It was first described in *S. cerevisiae* (20) as an integral component of the membrane that spans the membrane once with the N terminus inside the organelle and with the C terminus exposed to the cytosol. However, in *H. polymorpha*, Pex3p appears to be associated with the outer surface of the peroxisomal membrane without spanning it (25).

Selective turnover of membrane proteins has been observed in various cellular membranes. However, for peroxisomal membranes, this process has not been described before. So far, three mechanisms are known that result in the specific degradation of selected membrane proteins. Proteins of the plasma membrane (e.g. yeast Ste6p) first undergo ubiquitin-mediated internalization (endocytosis). Subsequently, the protein is transported via vesicle trafficking to the vacuole where it is degraded (26, 27). In the endoplasmic reticulum, membrane proteins to be degraded are marked with ubiquitin as well but subsequently are dislocated and degraded by the proteasome in the cytosol (28, 29). A well known example of this process is the degradation of defective cystic fibrosis transmembrane conductance regulator (a cAMP-regulated chloride channel), which leads to reduced lung function and, ultimately, death (30).

A third membrane protein degradation pathway occurs in bacteria, mitochondria, and chloroplasts and involves membrane-bound AAA-proteases that remove proteins from the membrane and subsequently degrade them (31). So far no peroxisomal membrane-bound proteases have been identified. However, general cellular degradation mechanisms like the ubiquitin/proteasome system may play a role in degradation of peroxisomal membrane proteins as well.

In *H. polymorpha*, peroxisomes are degraded individually, a process that is not inhibited by cycloheximide and apparently does not require protein synthesis. In *P. pastoris*, an identical process has been observed upon exposure of methanol-grown cells to excess ethanol (4). In *P. pastoris* but not in *H. polymorpha*, a second mode of pexophagy is also described, namely the glucose-induced bulk turnover of peroxisome clusters. This process designated microautophagy is dependent on protein synthesis (4).

At present, the first clues of the principles of the macro/microperoxophagy machinery in yeast become clear. Taking to-

---

<sup>1</sup> The abbreviations used are: GFP, green fluorescent protein; AO, alcohol oxidase; WT, wild type.

**FIG. 7. Kinetics of Pex3p levels in WT H. polymorpha.** Cells were shifted from glucose to methanol, and after incubation for 17 h, cells were incubated for 2 h in the presence or absence of MG-132. Blots were decorated with antibodies against Pex3p. Lane 1, glucose-grown cells; lane 2, 17-h methanol; lane 3, MG-132; and lane 4, + MG-132. Equal amounts of cells were loaded per lane.
gether the data of _S. cerevisiae_, _H. polymorpha_, and _P. pastoris_, the picture emerges that these processes overlap with other vacuolar sorting pathways, e.g. endocytosis, macroautophagy, and microautophagy and the cytoplasm to vacuole transport pathway (32–34). However, the selectivity of macroautophagy in _H. polymorpha_ is apparently organized at the initial stages of the process and most probably is not overlapping with other vacuolar protein sorting pathways (35, 36).

The selectivity of peroxisome degradation during macroautophagy in _H. polymorpha_ is manifested by the close sequestration of organelles to be degraded by various membrane layers prior to their uptake in the vacuole. Crucial for the understanding of the function of Pex3p removal in this process is information on how and at which stage of the process the protein is degraded. To this purpose, we analyzed two _H. polymorpha_ pdd mutants, namely pdd1, which is known to be affected in the first step of the pexophagy process, organelle sequestration, and pdd2, which shows normal sequestration but is impaired in the vacuole fusion process (15). Upon induction of macroautophagy, the turnover of Pex3p was fully prevented in pdd1 cells (14) but not in pdd2 cells. This led us to conclude that Pex3p degradation occurs prior to or during sequestration of peroxisomes. Apparently, Pex3p is not degraded in the vacuole but in the cytosol, a process that may directly or indirectly involve the function of the proteasome, because inhibition of the proteasome activity by MG-132 prevented Pex3p turnover.

The removal of Pex3p from the peroxisomal membrane seems to be a prerequisite to allow macroautophagy to proceed and requires the WT _H. polymorpha_ Pex3p. Indicative for this hypothesis are the findings that the replacement of HpPex3p by the bakers’ yeast homologue, ScPex3p, did not affect peroxisome biogenesis but inhibited organelle degradation. When GFP was fused to the C terminus of HpPex3p degradation of Pex3p-GFP and peroxisome turnover was significantly retarded. Possibly, correct exposure of specific domains in HpPex3p is important for the removal of the protein, a process that might be disturbed upon fusion of GFP to the C terminus. Together with the observation that initiation of macroautophagy does not require protein synthesis, this finding lends support to the view that modification/conformational changes or rearrangement reactions of already existing proteins have to occur to allow initiation of this degradation process. We speculate that the presence of GFP at the extreme C terminus of Pex3p may interfere with recognition of the domain that is involved in the initial stage of macroautophagy and/or blocks conformational changes/modifications of the protein that are necessary to allow its degradation. The importance of Pex3p removal to initiate peroxisome degradation is also in line with our previous observation that in cells in which HpPex3p is overproduced, selective peroxisome degradation is disturbed (37). Four hours after the induction of peroxisome degradation in a HpPex3p-overproducing strain, HpPex3p was still present, whereas in the WT control, HpPex3p was not detectable anymore at that time point (37). These data also indicate that the presence of HpPex3p inhibits peroxisome degradation.

Previously, we showed that Pex14p, one of the main docking proteins in the peroxisomal matrix protein import pathway, is essential to allow macroautophagy (5). The information that governed this process appeared to be located in the extreme N terminus of the protein (6). The data suggested that Pex14p may act as a kind of molecular switch that discriminated between organelle biogenesis and susceptibility to organelle degradation. Yet, it is unclear whether Pex3p and Pex14p take part in a joint machinery (e.g. in the tagging process of organelles destined for degradation) or serve completely different roles in the degradation process.

A clue in the order of Pex14p function and Pex3p removal may be deduced from the experiments in cells that lack Pex14p but in which peroxisome formation was restored by overproduction of the PTS1 receptor, Pex5p (strain _pex14_-P_AOX_PEX5<sup>mic</sup>). The reinduction of the organelles allowed us to address the fate of Pex3p upon exposure of the cells to excess glucose. Our data revealed that upon induction of macroautophagy in methanol-induced _pex14_-P_AOX_PEX5<sup>mic</sup> cells the level of Pex3p decreased with time. Thus, Pex3p is degraded independent from the function of Pex14p in macroautophagy. Essentially, similar experiments were performed with a strain that produced a truncated form of Pex14p that lacked the initial 64 N-terminal amino acids (PEX14-ΔN64) and thus could not degrade peroxisomes (6). Because in cells of the _PEX14-ΔN64_ strain Pex3p was degraded, we assume that in macroautophagy, the degradation of Pex3p occurs prior to the step that requires (the N terminus of) Pex14p.

In conclusion: this work has extended the list of multiple functions of Pex3p in peroxisome development (matrix protein import, membrane biogenesis, maintenance, and stabilization) to a role in peroxisome degradation. The molecular mechanisms of how the protein can serve these various mechanisms is largely unclear and is a topic of current investigations.

**Acknowledgments**—We thank Anne Koek, Anita Kram, and Klaas Sjollema for assistance in different parts of these studies.

**REFERENCES**

1. Subramani, S. (1998) _Physiol. Rev._ 78, 171–188
2. Klionski, D. J., and Osumi, Y. (1999) _Annu. Rev. Cell Dev. Biol._ 15, 1–32
3. Veenhuis, M., Douma, A. C., Harder, W., and Osumi, M. (1993) _Arch. Microbiol._ 159, 193–203
4. Tuttle, D. L., and Dunn, W. A., Jr. (1993) _J. Cell Sci._ 108, 25–35
5. Veenhuis, M., Komori, M., Salomons, F., Hilbrands, R. E., Hut, H., Baerends, J., Kiel, J. A., and Veenhuis, M. (1996) _FEBS Lett._ 388, 114–118
6. Bellu, A. R., Komori, M., van der Klei, I. J., Kiel, J. A. K. W., and Veenhuis, M. (2001) _J. Biol. Chem._ 276, 44570–44574
7. Baerends, R. S. J., Rasmussen, S. W., Hilbrands, R. E., van der Heide, M., Faber, K. N., Reuvelkamp, P. T., Kiel, J. A. K. W., Cregg, J. M., van der Klei, I. J., and Veenhuis, M. (1996) _J. Biol. Chem._ 271, 8878–8894
8. Van Dijken, J. P., Otte, R., and Harder, W. (1976) _Arch. Microbiol._ 111, 137–144
9. Baerends, R. S. J., Faber, K. N., Kram, M. A., Kiel, J. A. K. W., van der Klei, I. J., and Veenhuis, M. (2000) _J. Biol. Chem._ 275, 9886–9895
10. Samuels, J., Frischmann, E. F., and Cregg, J. M. (1995) _FEBS Lett._ 383, 114–118
11. Kiel, J. A. K. W., Rechinger, K. B., van der Klei, I. J., Salomons, F. A., Titorenko, V. I., and Veenhuis, M. (1999) _Yeast_ 15, 741–754
12. Bellu, A. R., Komori, M., van der Klei, I. J., and Veenhuis, M. (1995) _J. Biol. Chem._ 270, 2067–2070
13. Komori, M., Rasmussen, S. W., Kiel, J. A. K. W., Baerends, R. J. S., Cregg, J. M., van der Klei, I. J., and Veenhuis, M. (1997) _EMBO J._ 16, 44–53
14. Kiel, J. A. K. W., Rechinger, K. B., van der Klei, I. J., Salomons, F. A., Titorenko, V. I., and Veenhuis, M. (2000) _J. Biol. Chem._ 275, 12603–12611
15. Tito, G. J., Faber, K. N., Haima, P., Harder, W., Veenhuis, M., and Ab, G. (1994) _Curr. Genet._ 25, 305–310
16. Tan, X., Waterham, H. R., Veenhuis, M., and Cregg, J. M. (1995) _J. Cell Biol._ 128, 307–319
17. Kiel, J. A. K. W., Titorenko, V. I., Keizer, I., Harder, W., and Veenhuis, M. (1995) _J. Bacteriol._ 177, 357–363
18. Gibson, M. A. G., and Sudbery, P. E. (1988) _Yeast_ 4, 293–303
19. Salomons, F. A., Kiel, J. A. K. W., Faber, K. N., Veenhuis, M., and van der Klei, I. J. (2000) _J. Biol. Chem._ 275, 12603–12611
20. Veenhuis, M., van Dijken, J. P., Pilon, S. A., and Harder, W. (1978) _Arch. Microbiol._ 117, 163–168
21. Kiel, J. A. K. W., Keizer-Gunnink, I. K., Krause, T., Komori, M., and Veenhuis, M. (1995) _FEBS Lett._ 377, 434–438
22. Hicke, L. (1999) _Trends Cell Biol._ 9, 107–112
23. Kranz, A., Kinner, A., and Kelling H. (2001) _Mol. Biol. Cell._ 12, 711–720
24. Pomper, R. K., and Wolf, D. H. (1999) _Trends Biochem. Sci._ 24, 666–670
25. Benharouga, M., Haardt, M., Kartner, N., and Lukacs, G. I. (2001) _J. Cell Biol._ 153, 587–900

*The Role of Pex3p in Peroxisome Degradation* 42879
30. Skach, W. R. (2000) Kidney Int. 57, 825–831
31. Langer, T. (2000) Trends Biochem. Sci. 25, 247–251
32. Stromhaug, P. E., and Klionsky, D. (2001) Traffic 2, 524–531
33. Abeliovich, H., and Klionsky, D. (2001) Microbiol. Mol. Biol. Rev. 65, 463–479
34. Suzuki, K., Kirisako, T., Kamada, Y., Mizushima, N., Noda, T., and Oshumi, Y. (2001) EMBO J. 20, 5971–5981
35. Bellu, A. R., Kram, A. M., Kiel, J. A. K. W., Veenhuis, M., and van der Klei, I. J. (2001) FEBS Yeast Rev. 1, 23–31
36. Bellu, A. R., and Kiel, J. A. K. W. (2002) Microsc. Res. Technol., in press
37. Baerends, R. J. S., Salomons, F. A., Kiel, J. A. K. W., Van der Klei, I. J., and Veenhuis, M. (1997) Yeast 13, 1449–1463
38. Komori, M., and Veenhuis, M. (2000) Cell Biochem. Biophys. 32, 283–284