When glucose-starved cells are replenished with glucose, the key gluconeogenic enzyme, fructose-1,6-bisphosphatase (FBPase), is selectively targeted from the cytosol to the yeast lysosome (vacuole) for degradation. The glucose-induced targeting of FBPase to the vacuole for degradation occurs in cells grown under a variety of metabolic conditions. Immunoelectron microscopic studies demonstrate that the uptake of FBPase by the vacuole is mediated in part by an autophagic process. FBPase can be found on the vacuolar membrane and also at the sites of membrane invaginations. Furthermore, FBPase is associated with different forms of vesicles, which are induced to accumulate inside the vacuole. We have identified peroxisomes as the organelles that are delivered to the vacuole for degradation when cells are replenished with glucose. Ultrastructural studies indicate that peroxisomes are engulfed by the vacuole by an autophagic process, leading to the destruction of whole organelles in the vacuole. Furthermore, the galactose transporter (Gal2p) is also delivered from the plasma membrane to the vacuole for degradation in response to glucose. Gal2p is delivered to the vacuole through the endocytic pathway, as mutants defective in receptor-mediated endocytosis fail to degrade Gal2p in response to glucose.

Many pathways of protein degradation have been identified (1–3). Most short-lived normal proteins and abnormal proteins are degraded in the cytosolic, ubiquitin-dependent, or proteasome-dependent pathway (1–3), whereas most long-lived proteins are degraded in lysosomes (4, 5). Lysosomal degradation of cytosolic proteins is regulated by hormones and growth factors and is increased when cells are starved of nutrients (4–6). The enhanced lysosomal protein degradation requires the heat shock hsc73 protein and a targeting sequence present in many cytosolic proteins that are targeted to lysosomes for degradation such as RNase A and glyceraldehyde-3-phosphate dehydrogenase (7–10). Cytosolic proteins can also be taken up by autophagy such as RNase A and glyceraldehyde-3-phosphate dehydrogenase (11–13). The yeast vacuole plays an important role in the degradation of many cellular proteins (11–13). However, the mechanism responsible for the uptake of cytosolic proteins into the yeast vacuole is less understood. It was reported that the key regulatory enzyme in the gluconeogenesis pathway, fructose-1,6-bisphosphatase (FBPase), is inactivated in response to glucose (14–18). In addition to FBPase, many enzymes involved in the metabolism of carbohydrates (14, 19, 21), the tricarboxylic acid cycle (20), the control of flux of sugars (22–24), and the oxidation of fatty acids (25–27) are also inactivated by glucose. These enzymes include phosphoenolpyruvate carboxykinase (19), C-malate dehydrogenase (20), trehalose-6-phosphate synthase (21), trehalose-6-phosphate phosphatase (21), galactose transporter (22), maltose transporter (23), and alcohol oxidase and catalase (25–27). Some of these enzymes are cytosolic, while others are localized to the plasma membrane or in distinct organelles such as peroxisomes. The induction of these enzymes during glucose starvation serves to maximize the production of energy. When cells are replenished with fresh glucose, these enzymes are no longer needed and are inactivated. Given that a large number of enzymes involved in different metabolic pathways are inactivated by glucose, catabolite inactivation appears to play a critical role in the regulation of metabolism.

Studies using vacuolar proteinase-deficient mutants (pep4) have demonstrated that FBPase is targeted from the cytosol to the vacuole for degradation when glucose-starved cells are replenished with glucose (18). Using indirect immunofluorescence, we have found that FBPase displays an unusual punctate staining in the vacuole, which coincides with numerous particles that are induced to accumulate in the vacuole as seen by Nomarski microscopy. This prompted us to examine the import process at the ultrastructural level using immunoelectron microscopy. We have found that the uptake of FBPase into the vacuole is mediated by an autophagic process. FBPase can be found at the sites where membrane invaginations occur, indicating that FBPase is internalized by an autophagy of membranes structures. Inside the vacuole, FBPase is associated with different types of membranes, suggesting that other vesicles are also delivered to the vacuole for degradation in response to glucose. We have identified peroxisomes and the galactose transporter that are delivered to the vacuole for degradation when cells are replenished with glucose.

**EXPERIMENTAL PROCEDURES**

Strains and Antibodies—W3031 (MATa leu2-3, 112 ade2 his3 trp1-1 ura3-52) and pep4 (MATa leu2-3 112 ade2 his3 trp1-1 ura3-5 PEP4: TRP1) were obtained as described (18). Mutants defective in endocytosis such as end1-64 (MATa leu2-3 112, his4 trpl-1 ura3-52 bar1) were obtained from Dr. H. Riezman, University of Basel, Switzerland. Anti-FBPase antibodies were generated using purified FBPase. Anti-peroxisomal thiolase antibodies were produced by injecting rabbits using a protein A-thiolase fusion protein purified on an IgG affinity chromatography (Pharmacia Biotech Inc.). Anti-Gal2p was generated using N-terminal 14 amino acids conjugated to keyhole limpet
hemocyanin and injected into rabbits. Anti-plasma membrane ATPase antibodies were generated against C-terminal 12 amino acids conjugated to keyhole limpet hemocyanin and injected into rabbits to produce polyclonal antibodies.

Immunofluorescence—Cells were grown under various metabolic conditions to induce FBPase. Cells were cultured in YP containing 1% potassium acetate and 0.1% glucose for 48 h, YPD (2% dextrose) for 24 h, and adopted to YPO (2% oleic acid) for additional 24 h, YP containing 2% pyruvate for 48 h, YP containing 2% galactose for 48 h, and then transferred to glucose for 45 min. Cells were fixed and stained with anti-FBPase antibodies as described (18).

Immunoelectron Microscopy—Aliquots of A600 = 20 of cells were fixed in 3% formaldehyde, 0.5% glutaraldehyde with 0.25 M sorbitol added to the fixative. After dehydration in an ethanol series, the samples were infiltrated with LR White resin (Polysciences), embedded in fresh resin in gelatin capsules, and polymerized at 50 °C for 2 days. Ultra thin sections were mounted on Filmed nickel grids. Affinity-purified anti-FBPase antibodies were used at 1:5 dilution and anti-thiolase used at 1:300 dilution in overnight incubations at 4 °C. Grids were washed extensively with phosphate-buffered saline before incubation for 1 h with 15 nm gold-conjugated secondary antibodies (BioCell) at 1: 25 dilution. The sections were stained with uranyl acetate and lead citrate and examined in a Phillips 301 electron microscope.

Degradation of Total Cellular Proteins—Cells (A600 = 200) were precultured overnight, labeled in 10 ml yeast nitrogen base without sulfate and amino acids containing 2% oleic acid and 300 μCi of [35S]methionine and cysteine (DuPont) for 24 h. Cells (2 ml) were transferred to 2% glucose in yeast nitrogen base with excess unlabeled l-methionine and l-cysteine for 0, 45, 90, 180, and 300 min. Cells were centrifuged, and the supernatants (2 ml) were precipitated with 10% trichloroacetic acid with 50 μl of 2% bovine serum albumin at 4 °C. Degradation of long-lived proteins were calculated as the percentage release of radioactivity into a trichloroacetic acid-soluble fraction in the media. Samples were counted using a liquid scintillation counter.

Degradation of FBPase, Peroxisomes, and Gal2p—Cells (A600 = 200) were grown and labeled as described above. Cells (2 ml) were transferred to 2% glucose in yeast nitrogen base with excess unlabeled l-methionine and l-cysteine. Cell extracts were obtained and immunoprecipitation experiments were performed with 5 μl of anti-FBPase followed by protein A-Sepharose (Pharmacia). Proteins were separated on 10% SDS-PAGE gels, dried, exposed in PhosphorImager cassettes and quantified with a PhosphorImager (Molecular Dynamics). Degradation of FBPase was determined using t = 0 as 100%. Degradation of peroxisomes was followed with 2 μl of anti-thiolase antibodies. Samples were processed the same way as described for FBPase. To study the degradation of Gal2p, cells were grown in 2% galactose and transferred to glucose for 0–4 h.

RESULTS

FBPase Is Targeted into the Vacuole for Degradation—The role the vacuole plays in the degradation of FBPase in response to glucose was studied using isotopic strains differing only at the PEP4 gene. The PEP4 gene encodes proteinase A required for the maturation of proteinase B and proteinase C (11–13). Cells with a deletion of the PEP4 gene have reduced proteolytic activity to 30% of the wild type level (11–13) and allowed us to follow the uptake of FBPase by the vacuole. If FBPase was imported into the vacuole for degradation, a decrease in the level of FBPase would be expected in wild type cells harboring a functional PEP4 gene. In contrast, an accumulation of FBPase inside the vacuole would be detected in pep4 mutants due to a defect in vacuolar proteolysis. Hence, a PEP4-dependent degradation of FBPase would indicate that the vacuole participates in the degradation process. We have shown previously that FBPase is induced and accumulated in the cytosol when cells are grown in acetate. When glucose is added to the acetate-grown cells, FBPase is degraded rapidly in wild type cells. By contrast, FBPase degradation is delayed in pep4 mutants, suggesting that FBPase degradation requires the PEP4 gene. Using indirect immunofluorescence microscopic techniques, we have found that FBPase is in the cytosol in the acetate-grown pep4 cells (18). After a transfer of pep4 cells from acetate to glucose, FBPase is imported to the vacuole (18). Under this condition, the other cysolic proteins such as phosphoglycerate kinase and cytoplasmic invertase remain in the cytosol (18). To examine whether the glucose-induced import of FBPase into the vacuole was restrictive to certain growth conditions, we induced FBPase by growing cells in different metabolic conditions and transferring them to a medium containing fresh glucose. We have found that the addition of glucose induces the degradation of FBPase in wild type cells previously grown in acetate, oleic acid, pyruvate, and galactose (28). The induced degradation of FBPase is impaired in pep4 cells grown under all conditions, indicating that the degradation of FBPase requires the vacuolar PEP4 gene (28). To further confirm that FBPase import into the vacuole always occurs despite the growth conditions, we followed the subcellular localization of FBPase in wild type and pep4 cells grown under different metabolic conditions using indirect immunofluorescence. Fig. 1 shows that wild type cells grown in oleic acid contained high level of FBPase in the cytosol at t = 0 min (Fig. 1a). Following a transfer of wild type cells to glucose for 45 min, FBPase was completely degraded (Fig. 1b). pep4 cells grown in oleic acid also accumulated FBPase in the cytosol (Fig. 1c). However, transferring of pep4 cells to glucose for 45 min induced a redistribution of FBPase to the vacuole (Fig. 1d), consistent with our earlier report using the acetate-grown wild type and pep4 cells (18). Similarly, the galactose-grown pep4 cells accumulated FBPase in the cytosol (Fig. 1e). FBPase was targeted to the vacuole when pep4 cells were shifted from galactose to glucose for 45 min (Fig. 1f). A redistribution of FBPase from the
FBPase to the vacuole was also observed when pep4 cells were transferred from pyruvate (Fig. 1g) to glucose for 45 min (Fig. 1h). Thus, the glucose-induced import of FBPase into the vacuole for degradation always occurs regardless of growth conditions.

Glucose Induces Massive Protein Degradation and Autophagy—We have noticed that when FBPase was localized in the vacuole, FBPase displayed an unusual punctate staining which coincided with numerous particles as seen by Nomarski microscopy (Fig. 1, d, f, and h). These particles were induced in the glucose-replenished cells (Fig. 1, d, f, and h) as compared to the glucose-starved cells, which displayed a large but smooth vacuole (Fig. 1, c, e, and g). The accumulation of particles inside the vacuole was previously observed under Nomarski microscope using pep4 cells that have been transferred from acetate to glucose (18). However, the structures have not been examined at the ultrastructural level. To visualize these particles, we performed electron-microscopic studies on thin sections of pep4 cells. Fig. 2A shows that pep4 cells grown in acetate displayed a large vacuole. Some electron dense materials were occasionally observed inside the vacuole (Fig. 2A, a). Upon refeeding of the acetate-grown pep4 cells with glucose for 45 min, massive accumulation of vesicles inside the vacuole was observed (Fig. 2A, b). Under these conditions, the gross morphology of the cell seemed to be maintained. The accumulation of vesicles inside the vacuole was also observed when pep4 cells were transferred from galactose to glucose for 45 min (Fig. 2A, c) and from oleate to glucose for 45 min (Fig. 2A, d). As these vesicles occupied roughly 50% of the total cell volume, it represented the most profound changes that occurred during regrowth of cells in glucose. Some vesicles seemed to lose membrane boundaries and fused with other vesicles inside the vacuole. Since the number of vesicles was low in the glucose-starved cells, the accumulation of these vesicles inside the vacuole in glucose-replenished cells might result from an increased delivery of different organelles to the vacuole for degradation.

To assess the extent of protein degradation that occurred in the vacuole in response to glucose, we metabolically labeled proteins for 24 h in the glucose-starved cells to preferentially label long-lived proteins that were expressed during glucose starvation. We followed the degradation of long-lived proteins for 0–5 h in the presence of glucose and compared the rates of degradation of these proteins in the absence of glucose. Fig. 2B (a) shows that in the absence of glucose, cells degraded long-lived proteins at a slow rate. In a period of 5 h, only 1.5% degradation rate was observed. The addition of glucose accelerated the degradation rate 16-fold. The degradation rate increased linearly and reached 25% in 5 h (Fig. 2B, a). The induced degradation of long-lived proteins occurred primarily in the vacuole, as pep4 mutants blocked the degradation of long-lived proteins to 30% of the wild type level (Fig. 2B, b). In a chase of 5 h in glucose, only 7% of long-lived proteins were degraded in pep4 cells (Fig. 2B, b). Since the vacuole contains a variety of proteinases and that the deletion of the PEP4 gene affects only a subset of proteinases, the contribution of the vacuole could be greater than 70%. Bulk degradation of proteins requires the synthesis of new proteins, as cycloheximide inhibited the degradation of total long-lived proteins in the presence of glucose (Fig. 2B, c).

FBPase Enters the Vacuole by Autophagy—To examine the import of FBPase at the ultrastructural level, pep4 cells were glucose-starved, transferred to glucose for 45 min and processed for immunoelectron microscopic studies using affinity-purified anti-FBPase antibodies followed by secondary antibodies conjugated with 15 nm gold particles. Consistent with our immunofluorescence results, immunoelectron microscopic studies have demonstrated that the glucose-starved pep4 cells accumulated FBPase in the cytosol at \( t = 0 \) min (Fig. 3a). Transferring of pep4 cells to glucose for 45 min induced a localization of FBPase to the vacuole (Fig. 3b). At the ultrastructural level, the staining of FBPase with the vacuole appeared rather complex (Fig. 3, c and d). Some FBPase deco-
rated the vacuolar membrane (triangles), suggesting a direct binding of FBPase to components on the vacuolar membrane. Some FBPase decorated the regions where membrane invaginations occurred (arrows), suggesting that FBPase was internalized by an invagination and the formation of autophagic vesicles. We have observed one such site in Fig. 3c and two sites in Fig. 3d where FBPase was found. These membrane structures exhibited different electron densities, as some were stained heavier than the others. Furthermore, FBPase was observed with different forms of vesicles (arrows) inside the vacuole, suggesting that different vesicles were delivered to the vacuole for degradation under the conditions when FBPase was targeted to the vacuole for degradation. We have also observed a fraction of FBPase associated with electron dense materials without distinct membranous structures inside the vacuole. Fig. 3c shows that, among the 29 gold particles found in the vacuole, 4 gold particles decorated the vacuolar membrane, 3 at the sites where membrane invaginations occurred, 10 with vesicles inside the vacuole, and 12 with non-vesicles inside the vacuole. Similar distribution was observed in Fig. 3d. A total of 30 gold particles were found associated with the vacuole. Four gold particles decorated the vacuolar membrane, 3 at the sites of invaginations, 10 with vesicles inside the vacuole, and 13 with non-vesicles inside the vacuole.

Peroxisomes Are Delivered to the Vacuole for Degradation—Fig. 2A (b–d) and Fig. 3 (b–d) show that different forms of vesicles accumulated in the vacuole in response to glucose, suggesting that glucose induced a targeting of various organelles to the vacuole for degradation. In order to determine which organelles were targeted to the vacuole for degradation, we examined whether specific organelles were degraded in a PEP4-dependent manner by transferring wild type and pep4 cells to glucose for various periods of time. We first studied mitochondria and peroxisomes, two organelles that are known to be important for metabolic regulation (29–31). Degradation of these organelles was followed using antibodies directed against the mitochondrial hsp70, a specific marker for mitochondrial matrix proteins and also antibodies against 3-oxoacyl CoA thiolase, a specific marker for peroxisomal matrix proteins. Fig. 4A shows that wild type cells responded to glucose by degrading peroxisomes as evidenced by a decrease in the level of peroxisomal thiolase in response to glucose. Degradation of peroxisomes was defective in the pep4 deletion cells, indicating that the degradation of peroxisomes required the vacuolar PEP4 gene. By contrast, mitochondria showed no apparent degradation in either wild type or pep4 cells using antibodies directed against the mitochondrial hsp70. Similar observations were obtained using antibodies directed against the mitochondrial F1-ATPase (data not shown). Therefore, peroxisomes fulfilled the criteria as an organelle that was degraded in response to glucose in a PEP4-dependent manner.

The kinetics of the degradation of peroxisomes was different from that of FBPase. In wild type cells, FBPase is degraded with a half-life of 30 min in response to glucose. In addition, no FBPase can be detected after 1 h of shift to glucose. Fig. 4B shows that the kinetics of the degradation of peroxisomes was slower than that of FBPase. Peroxisomes were degraded with a half-life of 1.5 h in wild type cells (Fig. 4B, a). Furthermore, 30% of peroxisomal proteins remained after 5 h of chase in glucose (Fig. 4B, a). The significance of these differences is not clear. The remaining peroxisomes may be important for the synthesis of new peroxisomes if cells are regrown in a medium that requires the proliferation of the entire organelles. The degradation of peroxisomes required the PEP4 gene, as pep4 cells blocked the degradation of peroxisomes with a prolonged half-life of more than 10 h (Fig. 4B, a). The addition of cycloheximide in the presence of glucose inhibited the degradation of peroxisomes with a half-life of more than 10 h, indicating that the degradation of peroxisomes required the synthesis of new proteins (Fig. 4B, b).
To study whether peroxisomes were delivered to the vacuole for degradation, we performed immunoelectron microscopic studies on thin sections of pep4 cells that have been transferred to glucose for 45 min. Fig. 4C shows that, despite a long half-life, uptake of peroxisomes by the vacuole could be observed at 45 min of glucose shift (Fig. 4C, a–c). Fig. 4C(a) shows that a total of eight peroxisomes were seen in the whole cell, with four localized inside the vacuole and four in the cytoplasm. Peroxisomes were internalized by an autophagic process, as indicated by the localization of peroxisomal thiolase inside membranous structures indistinguishable from the ones in the cytoplasm. If a direct fusion between peroxisomes and the vacuole had occurred, a mixing of the peroxisomal matrix proteins and a distribution of the peroxisomal thiolase in the matrix of the vacuole would be expected. The engulfment of peroxisomes by the vacuolar membrane could be observed in several thin sections of pep4 cells (arrows in Fig. 4C, b and c).

The Galactose Transporter Is Delivered to the Vacuole by Endocytosis—As shown in Fig. 4C, peroxisomes contributed to some of the vesicles that accumulated inside the vacuole when cells were shifted to glucose. However, there were other membranous structures that could not be stained with antibodies directed against peroxisomes, suggesting that other organelles were also delivered to the vacuole for degradation. The possibility that endosomes played a role was examined using antibodies directed against two plasma membrane proteins, the galactose transporter (Gal2p) and the plasma membrane ATPase. We determined whether any of these plasma membrane proteins showed a glucose-induced degradation and whether their degradation required the PEP4 gene by following the degradation of these proteins in wild type and pep4 cells shifted to glucose for 0–4 h. Fig. 5A shows that Gal2p, like FBPase and peroxisomes, was degraded in wild type cells in response to glucose. The degradation of Gal2p was impaired in pep4 cells, indicating that the degradation of Gal2p required the vacuolar PEP4 gene. Under the same conditions, the plasma membrane ATPase showed no degradation in either wild type or pep4 cells, indicating that selective degradation of plasma membrane proteins occurred when cells were transferred to glucose. Fig. 5B shows that the degradation of Gal2p was inhibited by the addition of cycloheximide in the presence of glucose (Fig. 5B, b), indicating that the degradation of Gal2p required the synthesis of new proteins. In addition, the degradation of Gal2p was sensitive to the carbon sources added to the medium. For example, transferring of wild type cells to sucrose, a disaccharide consisting of fructose and glucose, also induced the degradation of Gal2p (Fig. 5B, c). By contrast, transferring of wild type cells to galactose (Fig. 5B, d) or acetate (Fig. 5B, e) had no effect on the degradation of Gal2p.

The degradation of Gal2p is dependent on the endocytic pathway. In Saccharomyces cerevisiae, the pheromone α-factor binds to the α-factor receptor on the surface of a cells and triggers an internalization of the receptor complex (32–34). Early endosomes containing the internalized receptor complex mature into late endosomes, which fuse with post-Golgi vesicles and deliver the receptor complex to the vacuole for degradation (32–34). Mutants defective in receptor-mediated endocytosis have been identified. These end mutants show temperature sensitivity for growth. At the non-permissive temperature, they are defective not only in the endocytosis of
α-factor but also the accumulation of lucifer yellow in the vacuole (32–34). end1 is identical to vps11 and pep5 (33, 35). They contain no recognizable vacuole (33). end3 and end4 affect the internalization step of the endocytic pathway (34). To determine whether Gal2p was degraded in the vacuole through the endocytic pathway, the degradation of Gal2p was examined in end mutants. Fig. 5B shows that Gal2p was degraded in response to glucose in wild type cells at 37 °C. The kinetics of Gal2p degradation was slightly faster at 37 °C than at 30 °C in wild type cells. At the non-permissive temperature, end1, end2, end3, and end4 all showed defects in degrading Gal2p (Fig. 5B, g–j). Representative results of the kinetics of Gal2p degradation are shown in Fig. 5C. In wild type cells, Gal2p was degraded with a half-life of 1 h after glucose readdition. The degradation of Gal2p was blocked in pep4 cells with a prolonged half-life of more than 10 h (Fig. 5C, a). As was the case for FBPase, peroxisomes, and long-lived proteins, the addition of cycloheximide inhibited the degradation of Gal2p with a half-life of longer than 10 h (Fig. 5C, b). Delayed degradation of Gal2p with half-lives of longer than 10 h was also observed in end3 and end4 mutants (Fig. 5C, c), indicating that Gal2p was degraded in the vacuole through the endocytosis pathway.

DISCUSSION

When glucose-starved cells are replenished with glucose, massive protein degradation is induced. In a period of 5 h, 25% of total long-lived proteins are degraded. The increased degradation occurs primarily in the vacuole as pep4 cells block the induced degradation in response to glucose. During this time, an accumulation of autophagic vesicles inside the vacuole is also observed. As these vesicles display great heterogeneity, different organelles may be delivered to the vacuole for degradation when glucose is added to the medium. Consistent with that observation, we have identified three pathways of protein targeting into the vacuole for degradation using markers of the cytosol, peroxisomes, and the plasma membrane. Fig. 6 illustrates that FBPase, peroxisomes, and Gal2p are all targeted to the vacuole for degradation when cells are transferred to glucose. These three pathways of protein degradation share several common features. (a) The degradation of FBPase, peroxisomes, and Gal2p depend on the vacuolar PEP4 gene. (b) The degradation of FBPase, peroxisomes, and Gal2p all occur in a glucose-regulated manner. (c) The synthesis of new proteins is
During the formation of AV, cytosolic proteins can be engulfed and enclosed into AV in a non-selective manner. These AV then fuse with lysosomes, resulting in the destruction of AV with enclosed cytosolic proteins in lysosomes (40, 41). Both microautophagy and macroautophagy have been shown to deliver cytosolic proteins to lysosomes in a non-selective fashion (4, 5, 36–39). Based on our immunoelectron microscopy observations that FBPase can be found at the sites where membrane invaginations occur, FBPass appears to be taken up by microautophagy at least during the late stage of the internalization and degradation process. However, a fraction of FBPass is associated with electron-dense materials without distinct membranous structures. We consider three possibilities that may explain this observation. (a) FBPass is taken up by microautophagy by an invagination and engulfment of the vacuolar membrane. The vesicles containing FBPass are pinched off and moved into the matrix. The vesicles are subsequently broken by lipase to release FBPass to the vacuolar matrix for further degradation by proteases in the vacuolar matrix. The staining of FBPass in the matrix may represent the FBPass that has been released to the matrix, but has not yet been degraded by proteases. (b) FBPass is transported into the lumen of a different type of vesicles. After a fusion of the vesicles with the vacuole, FBPass can be released directly into the lumen of the vacuole for degradation. This may account for the staining of FBPass in the matrix. This model predicts that an intermediate compartment is used to deliver FBPass to the vacuole for degradation. Since no such vesicles can be observed at the 45-min time point, this process may occur at earlier time points. (c) FBPass is taken up by a direct translocation. FBPass may bind to a receptor protein on the vacuolar membrane and is translocated into the lumen of the vacuole for degradation. Unfolding of FBPass by heat shock proteins may occur to facilitate the transport of FBPass to the vacuole. This model would be similar to the uptake of RNase A by lysosomes in mammalian cells (8–10).

FBPass are engulfed by the vacuole also by autophagy, resulting in the degradation of whole organelles in the vacuole. In P. pastoris, both microautophagy and macroautophagy can operate to degrade peroxisomes depending on metabolic conditions (26). Transferring of methylotrophic P. pastoris to glucose induces the degradation of peroxisomes by microautophagy, whereas transferring of methanol-grown cells to ethanol induces the degradation of peroxisomes by macroautophagy (26). The protein synthesis inhibitor, cycloheximide, blocks the microautophagy induced by glucose, but not macroautophagy induced by ethanol (26). In our studies, the degradation of peroxisomes is inhibited by cycloheximide. It is likely that peroxisomes are taken up by microautophagy when S. cerevisiae are transferred to glucose. This is consistent with our immunoelectron microscopy studies that peroxisomes are internalized by an engulfment by the vacule during regrowth of cells in glucose.

It has been reported that FBPass is stabilized in the proteosome mutants (42). Recent evidence has indicated that the SSV7 gene required to maintain the integrity of the vacuole is identical to the DOA4 gene involved in the proteosome degradation pathway (43, 44). ss7 mutant was originally isolated as mutant defective in vacuolar biogenesis (43). Vacuolar proteases such as CPY is missorted to the cell surface in ss7 mutants (43). The stabilization of FBPass in the proteosome mutants may result from an indirect effect of mutations that impair the biogenesis of the vacuole.

Catabolite inactivation of FBPass was described by Gancedo in 1971 (14). We show evidence that catabolite inactivation of FBPass is mediated by a selective targeting of FBPass to the vacuole for degradation. Catabolite inactivation of Gal2p was
reported by Matern and Holzer in 1977 (22). We show that Gal2p is delivered to the vacuole for degradation. Inactivation of peroxisomes mediated by autophagy was reported in H. polymorpha, P. pastoris, and C. boidinii (25–27). Consistent with these findings, we demonstrate that peroxisomes are targeted to the vacuole for degradation by autophagy when S. cerevisiae are transferred to glucose. Therefore, protein targeting and degradation by the vacuole are responsible for the glucose-dependent inactivation of these enzymes, and the vacuole degradation plays an important role in the regulation of the flux of sugars and the metabolism of carbohydrates and fatty acids.

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