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Peroxisomal assembly: membrane proliferation precedes the induction of the abundant matrix proteins in the methyiotrophic yeast Candida boidinii

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Summary

Peroxisomes are massively induced when methyiotrophic yeasts are cultured in medium containing methanol. These organelles contain enzymes that catalyze the initial steps of methanol assimilation. In Candida boidinii, a methyiotrophic yeast, the peroxisomal matrix (internal compartment) is composed almost exclusively of two proteins, alcohol oxidase and dihydroxyacetone synthase; catalase is present in much lower abundance. Monoclonal and polyclonal antibodies are available against peroxisomal matrix and membrane proteins. These were utilized to correlate the induction of specific proteins with the morphological changes occurring during peroxisomal proliferation. Cells cultured in glucose-containing medium contain two to five small microbodies, which are identifiable by catalase staining and immunoreactivity with a monoclonal antibody against PMP47, an integral peroxisomal membrane protein. Three stages of proliferation can be distinguished when cells are switched to methanol as the carbon source. (1) There is an early stage (within 1 h) in which several peroxisomes develop from a pre-existing organelle. This is accompanied by an increase in catalase activity and an induction of PMP47, but no detectable induction of alcohol oxidase or dihydroxyacetone synthase is observed. (2) From 1 to 2.5 h there is further division of these microbodies until up to 30 small peroxisomes generally are present in each of one or two clusters per cell. Induction of alcohol oxidase, dihydroxyacetone synthase and PMP20, a protein that is distributed in the matrix and membrane, is detectable during this time. Serial sections reveal that some peroxisomes remain uninduced while others undergo proliferation. Such sections also show no obvious connections between peroxisomes within clusters. (3) After 2.5 h there is a decrease in the number of peroxisomes per cell (caused at least in part by the movement of organelles into buds) but an increase in volume per peroxisome, until a steady state is reached by 5-10 h.

Key words: peroxisomes, methyiotrophic yeast, Candida boidinii.

Introduction

Microbodies (peroxisomes, glyoxysomes and related organelles) are found in virtually all eukaryotic cells and promote a variety of diverse oxidations and metabolic conversions (Fahimi and Sies, 1987). In fungi the proliferation, enzyme composition and metabolic function(s) of microbodies can readily be manipulated by changes in the growth environment (Veenhuis and Harder, 1987), and this characteristic permits a detailed analysis of peroxisomal proliferation that may be applicable to peroxisomal biogenesis in general. Like microbodies in plant and animal systems, the proteins of the yeast peroxisomal matrix are synthesized as soluble precursors that generally do not contain amino-terminal extensions (Lazarow and Fujiki, 1985); the sorting signal may often reside in the extreme carboxy terminus (Gould et al. 1988). Assembly is dependent on energy (Bellion and Goodman, 1987; Imanaka et al. 1987). Morphological evidence obtained so far strongly suggests that these organelles, at least under conditions that cause proliferation, develop by fission from existing microbodies following induction and import of substrate-specific enzymes. For example, in Hansenula polymorpha, a well-studied methyiotrophic yeast in which microbody proliferation is massively induced after transfer from glucose containing medium, single peroxisomes swell to 80 times their original volume and subsequently multiply by the separation of small organelles from mature ones (Veenhuis et al. 1979).

Like other methylotrophs, the methanol-induced peroxisomes of Candida boidinii contain the enzymes that catalyse the initial steps of methanol utilization, alcohol oxidase and dihydroxyacetone synthase (Goodman, 1985; Veenhuis and Harder, 1987). These two proteins account for almost the entire mass of the organelle (Goodman et al. 1984). Catalase, which destroys the hydrogen peroxide produced by alcohol oxidase, is also present in this compartment in much smaller amounts (Roggenkamp et al. 1975). The membrane proteins from these peroxisomes...
have been described; the most abundant have apparent molecular masses (on SDS-containing gels) of 20, 31, 32 and 47 K (K = 10^3 M_\text{r}) and are termed PMPs (peroxisomal membrane proteins). Monoclonal antibodies have been raised against PMP20 (Garrard and Goodman, 1989), a peripheral membrane protein, and PMP47, an integral membrane protein (Goodman et al. 1986).

The present report describes a detailed study of microbody proliferation in C. boidinii. The availability of antibodies has permitted for the first time a correlation of early morphological events with the induction of specific peroxisomal matrix and membrane proteins. We find that proliferation of the peroxisomal membrane precedes the induction of the abundant matrix proteins. Three phases of microbody proliferation in C. boidinii can be distinguished on the basis of morphology, immunolocalization, and induction of specific proteins. We also report that PMP47 is a useful marker of repressed as well as induced microbodies.

Materials and methods

Organism, growth and cell harvesting

*Candida boidinii* (ATCC no. 32196) was used in all experiments. The organism was grown in shaker flask cultures at 30 °C in mineral medium (van Dijken et al. 1976). For the experiments in which Western blotting was coupled with electron microscopy, cells were cultured in medium containing 2.5% glucose, harvested in mid log phase (A_600 of 3–5), washed once in distilled water and resuspended in medium containing 0.5% methanol at an A_600 of 1.0. At the indicated times, cell samples were withdrawn, drawn twice in water, and 50 A_600 units of cells were processed each for electron microscopy, gel electrophoresis and immunoblotting, and enzyme assays as described below.

In some experiments immunoelectron microscopy and serial sectioning were performed on cells that had been extensively preincubated on 0.5% glucose as described previously (Veenhuis et al. 1979) before dilution into medium containing 0.5% (v/v) methanol. The pattern of microbody proliferation was identical to that found in the previous method, although the lag time before changes occurred was shortened.

Enzyme and protein assays

Cell pellets were frozen at −70°C until assays were performed; 300 μl of ice-cold buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride) and 0.3 g acid-washed glass beads were added to the frozen cell pellets, and they were hand vortexed at 4°C for 20 min to lyse the cells. The liquid was removed from the beads and centrifuged in a microfuge for 3 min to remove debris and unlysed cells. Enzyme activities of alcohol oxidase, dihydroxyacetone synthase, and catalase, as well as determination of protein concentrations, were performed as previously described (Goodman, 1985).

Immunoblotting

Cell pellets were resuspended in 1 ml of 5% trichloroacetic acid and kept on ice for at least 15 min to ensure denaturation of all proteins. Suspensions were centrifuged for 15 min in a cold microfuge, resuspended in 250 μl Laemmli sample buffer (Laemmli, 1970) without Bromphenyl Blue, and frozen until later use. Thawed samples were neutralized with a small volume of NaOH and then 0.5 g of acid-washed glass beads was added. Samples were lysed by hand vortexing for 20–30 min and the lysates were removed from the beads. They were boiled for 3 min and then centrifuged for 3 min in a microfuge; the pellets were discarded. Protein concentrations of all lysates were determined; they were typically 9 mg ml⁻¹.

Samples were electrophoresed through SDS-containing gels (Laemmli, 1970; as modified by Goodman et al. 1986), transferred to nitrocellulose, and probed with monoclonal or polyclonal antibodies (Towbin et al. 1979). To quantitate relative concentrations of peroxisomal proteins in the lysates, a standard curve was constructed for each protein by varying the volume of the induced lysate that was applied to the gels. Preliminary blots were performed to determine the optimal amount of each lysate to apply in order that the signal would fall within the standard curve for each peroxisomal protein. In a typical case, this varied from 120 μg of the repressed lysates to 6 μg of the fully induced lysates.

Dilutions of antibody used in these blots were 1:500 for the polyclonal G358 anti-alcohol oxidase antisera (Goodman et al. 1984), and 1:50, 1:100 and 1:200 for the monoclonal IVA7 (anti-PMP47), IIIC1 (anti-PMP20) and IIB5 (anti-dihydroxyacetone synthase) antibodies (ascites fluids were used) (Goodman et al. 1986).

Electron microscopy and immunocytochemistry

Cytochemical demonstration of catalase activity was performed by the diaminobenzidine-based method described previously (Veenhuis et al. 1976). For the detection of PMP20 and PMP47 by immunocytochemical means intact cells were fixed in 3% (v/v) glutaraldehyde in 0.1 m sodium cacodylate buffer, pH 7.2, for 90 min at 0°C, dehydrated in a graded ethanol series and embedded in Lowicryl K4M (Zagers et al. 1986). Polymerization was set at −35°C by ultraviolet light. Immunogold labeling of alcohol oxidase, dihydroxyacetone synthase and both PMPs was performed on ultrathin sections using specific antibodies against these proteins and protein A/gold (Slott and Geuze, 1984). Gold particles were prepared by the citrate method (Frens, 1973). For studies of the cell morphology, whole cells were fixed in 1.5% (w/v) KMnO_4 for 20 min at room temperature. Postfixation was performed in a solution of 1% (w/v) OsO_4 + 2.5% (w/v) K_2Cr_2O_7 in the same cacodylate buffer for 90 min at room temperature. After fixation/postfixation, and also after cytochemical staining experiments, the sections were postfixed in 1% (w/v) uranyl acetate in distilled water for 8–16 h, dehydrated in a graded ethanol series and embedded in Epon 812. Ultrathin sections were cut with a diamond knife and examined in a Philips EM 300.

The average number of peroxisomes per cell and their relative volume fraction was determined on thin sections of KMnO_4-fixed cells as described previously (Veenhuis et al. 1979).

Results

Localization of PMP20 and PMP47

Electron micrographs of *Candida boidinii* cultured for several generations in medium containing glucose or methanol as the only carbon source are shown in Fig. 1A and B, respectively. While microbodies are not easily identifiable in cells grown on glucose, they are clearly seen in those induced by methanol. Immunocytochemical experiments performed on thin sections of methanol-cultured cells with polyclonal antibodies against the matrix protein alcohol oxidase (Goodman et al. 1984) confirmed its intra-peroxisomal location (Fig. 1C). A similar pattern was observed for dihydroxyacetone synthase, the other abundant matrix protein (data not shown). Fig. 1D shows a representative immunoelectron micrograph utilizing a monoclonal antibody against PMP20. While there is immunoreactivity over the peroxisomal membrane in agreement with biochemical data indicating that PMP20 is a peripheral membrane protein (Goodman et al. 1986), there is labeling over the matrix as well. We suspect the fraction of PMP20 localized to the matrix in this figure is bound to internal peroxisomal structural components that copurify with membranes upon organellar lysis. PMP20 is encoded by two genes (see Garrard and Goodman, 1989); it is possible that the corresponding proteins have different affinities for components of the membrane.

In contrast to PMP20, the localization of PMP47 is
Fig. 1. A. *C. boidinii* from the exponential growth phase on glucose. Arrow indicates a small microbody (peroxisome) that is occasionally seen. B. *C. boidinii* from the late exponential growth phase on methanol; peroxisomes (p) are apparent in electron-dense clusters. C,D,E. Immunoelectron micrographs in which cells grown on methanol are incubated with antibodies against alcohol oxidase (serum G358), PMP20 (monoclonal Ab IIIC1), or PMP47 (monoclonal Ab IVA7), respectively, followed by incubations in protein A/gold. n, nucleus; v, vacuole. Bar, 0.5 μm.

clearly limited to the peroxisomal membrane (Fig. 1E), consistent with its integral association with isolated peroxisomal membranes (Goodman et al. 1986).

**Microbodies in glucose medium and early changes following methanol shift**

In order to define the process of microbody proliferation in *C. boidinii* and to correlate the induction of specific matrix and membrane proteins with this process, cells growing in glucose-containing medium were harvested in mid-log phase and diluted into methanol-containing medium. Samples were taken at subsequent times for morphological study as well as for measurement of levels of specific proteins by quantitative Western analysis and enzyme assays. Immunoelectron microscopy was performed in similar but independent experiments. Observations on ultrathin serial sections of KMnO$_4$-fixed cells reveal that glucose-grown cells generally contain two to five peroxisomes, characterized by the presence of catalase (Fig. 2A). This number appears to be dependent on environmental conditions such as growth phase and cell cycle; more are found in cells during budding. These organelles were rounded or slightly irregular in shape, measured approximately 0.1–0.2 μm, lacked crystalline inclusions and were randomly distributed through the cell. Fig. 2B indicates that such organelles can also be identified by immunoelectron microscopy utilizing a monoclonal anti-PMP47 antibody. Western blotting revealed a very low but detectable concentration of PMP47 in these repressed cells, representing about 0.5% of that in fully induced cells (Fig. 3).

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Fig. 2. Early stages of peroxisomal proliferation. A,B. Peroxisomes in glucose-grown cells visualized by catalase staining (arrow) or by reactivity with anti-PMP47 monoclonal antibody, respectively. C,D. 1.5 h after the shift to methanol; C shows the typical irregularly shaped, interwoven organelles typical at this time in an Epon-embedded sample. The inset demonstrates budding during this phase. ×40,000; D demonstrates the lack of reactivity with anti-PMP20 monoclonal antibody. E,F. 2.5 h after the shift. A sample embedded in Epon is shown in E; a parallel sample processed for immuno-electron microscopy and treated with anti-PMP47 antibody is shown in F. G,H. Peroxisomes after 3.5 h. An Epon-embedded sample is shown in G; the parallel sample treated with anti-PMP20 antibody is shown in H. PMP20 reactivity is not seen before this time. m, mitochondrion; n, nucleus; p, peroxisome; v, vacuole. Bar, 0.5 μm.

No alcohol oxidase, dihydroxyacetone synthase or PMP20 could be visualized in these organelles with antibodies (data not shown), consistent with the lack of detection of these proteins by immunoblotting.

Although cells after 30 min of incubation in methanol were not sampled in this experiment, peroxisomes observed as shortly as 15 min after incubation in similar experiments showed noticeable deformation of shape. Serial sectioning revealed that the organelles appeared flatter, and that one or more extensions, often 0.5 μm in
The experiment was repeated twice with similar results. See Materials and methods for further details. (□) AO, alcohol oxidase; (○) DHAS, dihydroxyacetone synthase; (▲) PMP47; (●) PMP20. Inset: growth curve during the experiment, determined by light scattering.

Stage II: active microbody proliferation
By 1.5 h after the shift of cells to methanol clusters of several small microbodies were apparent (Fig. 2C). They were still very irregular in shape and were often seen interweaving among themselves; organelar budding still could be seen (inset to Fig. 2C). These clusters typically contained 5–10 peroxisomes.

During this initial period of peroxisomal membrane proliferation a fivefold increase in the concentration of PMP47 was detected (Fig. 3). PMP47 was detected by immunolabeling over peroxisomal membranes during every stage of microbody proliferation; labeling of neighboring membranes that resemble the endoplasmic reticulum was never seen. In contrast, at 1.5 h post-shift alcohol oxidase and PMP20 were not detectable by Western analysis (Fig. 3), enzyme assay (Fig. 4) or immunoelectron microscopy (Fig. 2D; and unpublished data). There was also no evidence for the presence of dihydroxyacetone synthase by immunoelectron microscopy (not shown) nor by immunoblots (Fig. 3). Enzymatic activity of dihydroxyacetone synthase could be detected in lysates of glucose-grown cells, which did not increase during this initial induction period; it probably represents an immunologically distinct and constitutively expressed enzyme or other interfering activity found elsewhere in the cell. Levels of proteins below 0.1% of fully induced levels would be detectable by Western blotting, on the basis of standard curves. Thus, we conclude that these early morphological changes were not accompanied by induction of the major matrix proteins. In contrast, the activity of catalase, a minor protein by mass (Goodman, 1985) increased approximately twofold (Fig. 4A).

Active organelar proliferation continued for another hour (to 2.5 h post-induction), such that clusters of 30 organelles were often seen at that time. The organelar clusters contained microbodies that appeared quite irregular in shape, and remained tightly interwoven. There was usually only one such cluster per cell, although two were occasionally seen. Serial sectioning failed to show visible connections between most microbodies in a cluster; however, isolated single unproliferated peroxisomes were often seen in these sections, suggesting that not all of the microbodies originally present in the glucose-grown inclusions proliferated (data not shown). Such single peroxisomes could be immunolabeled with PMP47 but not an induced matrix protein such as alcohol oxidase, confirming its uninduced state. Clusters presumably arose from one or two single peroxisomes that multiplied and adhered to each other. Such adherence may be weaker at later times of induction, since the individual organelles eventually became rounder and more separated (Fig. 2G).

By 2.5 h the matrix proteins alcohol oxidase and dihydroxyacetone synthase, and PMP20, could be observed on Western blots, although at extremely low amounts (1.8, 0.5 and 2.0% of their fully induced respective concentrations). In contrast, the volume of peroxisomes by this time had increased several-fold and was 12% of the fully induced (10 h) value (Fig. 5). These data indicate that peroxisomal proliferation at early time points cannot be driven by import of the major matrix proteins. Although it remains possible that early proliferation occurs as a result of import of unknown proteins, it is more likely that expansion of the peroxisomal membrane (by yet unknown mechanisms) accompanied by assembly of membrane pro-
tein (such as PMP47) is a primary event in peroxisomal development. Fig. 2F shows the localization of PMP47 at the 2.5 h time point. Since there was no evidence for specific labeling of other membranes such as the endoplasmic reticulum that could often be seen, PMP47 probably inserts directly into the growing organelles; pulse-chase experiments will be required to confirm this interpretation.

Stage III: enlargement of microbodies
By 2.5 h the number of microbodies had reached a maximum value (Fig. 6). After this point there was a decrease in the number of organelles, although the volume of each peroxisome rapidly increased. A typical example of the microbody proliferation after 3.5 h of cultivation in the new environment is shown in Fig. 2G. This stage of peroxisomal development was also accompanied by a strong induction of alcohol oxidase and dihydroxyacetone synthase as well as PMP20 (Fig. 3). Although PMP20 showed a greater rate of induction than that of alcohol oxidase and dihydroxyacetone synthase in this figure, this difference was not consistently seen.

The increase in activity of alcohol oxidase mirrored the increase in the concentration of this protein during the entire time course (Fig. 4B). Since oligomerization and binding of cofactor is necessary for activity, and these steps are assumed to occur within the peroxisome (Goodman et al. 1984), these data suggest that peroxisomes are capable of assembling peroxisomal proteins at all times during the induction of alcohol oxidase, although the capacity for this process may be quantitatively different at different stages of induction. The same behavior was found for dihydroxyacetone synthase (not shown).

The segregation of peroxisomes between mother and daughter cells appears to be responsible for the reduction in number of peroxisomes per cell at the late stage of proliferation. Peroxisomes were often found in the buds (Fig. 5A), and a cluster was occasionally seen being pinched off by the neck of the budding cell. Although this process occurred during earlier stages of proliferation, the segregation of organelles was offset by the massive increase in number of organelles. No evidence for fusion of small microbodies into larger ones was ever seen. By 10 h following induction the morphology of peroxisomes strongly resembled cells in steady-state growth in methanol (Fig. 5B). This figure (5B) also demonstrates that small organelles were often apparent in the peroxisomal cluster (arrows), presumably after separating from the mature microbodies.

Discussion
While peroxisomes were originally thought to bud from the endoplasmic reticulum (de Duve and Baudhuin, 1966), there is ample evidence that they are in fact derived from pre-existing organelles, or from a 'peroxisomal reticulum'. Peroxisomal matrix proteins are inserted directly into peroxisomes from cytosolic precursors (Lazarow and Fujiki, 1985). While the assembly of peroxisomal membrane proteins has been characterized to a far lesser extent, there is growing evidence that at least some
membrane proteins follow a post-translational pathway (Fujiki et al. 1984; Koester et al. 1986; Suzuki et al. 1987). Some differences in sorting pathways of matrix and membrane proteins are expected, however, since peroxisomes in patients with Zellweger disease appear to contain membrane proteins lacking matrix proteins (Santos et al. 1988a,b). Although the biochemical evidence for peroxisomae ex peroxisomis (inspiration attributed to C. de Duve) is strong, morphological support is required to strengthen this hypothesis. Using serial sectioning, Gorgas has demonstrated a peroxisomal reticulum in lipid-synthesized epithelia from several avian and mammalian sources (Gorgas, 1987). Such a reticulum has also been seen in regenerating rat liver (Yamamoto and Fahimi, 1987). Small organelles, identifiable as peroxisomes with a catalase stain, have been seen in systems when peroxisomal proliferation is suppressed (Lazarow et al. 1987; Veenhuis et al. 1979). It has been suggested that during adaptation of cells to a new environment that induces the proliferation of microbodies, all newly formed organelles are derived by growth and division of pre-existing peroxisomes (Veenhuis and Harder, 1987). The aggressive proliferation of microbodies in yeasts allowed us to study this process in fine detail. We were able to dissect proliferation into three successive steps (Fig. 7). Within an hour after incubation in methanol, small peroxisomes became elongated and formed processes from the peroxisomal body. These processes became the source of new peroxisomes as the original microbodies underwent fission. This behavior was also observed in epithelial cells (Gorgas, 1987). During these early steps there was no detectable induction of PMP20 or of the two matrix proteins found in the fully induced organelle; these proteins are clearly not required for these early steps in proliferation. The increase in PMP47 concentration mirrors the growth of the peroxisomal membrane during this time. Catalase activity also increases during these early stages of proliferation. This may indicate a requirement for this enzyme prior to the appearance of alcohol oxidase or may reflect simply a rapid derepression of catalase upon the depletion of glucose from the medium. Proliferation continues for two or three more hours. During this time alcohol oxidase, dihydroxyacetone synthase, and PMP20 can be detected, although at very low concentrations. Whether specific proteins are imported into the matrix during this time that are involved solely with proliferation is not known. As the number of peroxisomes reaches a maximum, the volume of the nascent cluster increases dramatically. Not surprisingly, this enlargement phase is also accompanied by a large rate of synthesis (and import) of alcohol oxidase, dihydroxyacetone synthase and PMP20. As enlargement proceeds, the number of peroxisomes per cluster gradually decreases as the rate of segregation of peroxisomes into cell buds overcomes the rate of generation of new organelles, until a steady state is reached.

It is interesting that the early steps in proliferation of microbodies of Candida boidinii are different from another methylotrophic yeast, Hansenula polymorpha. In H. polymorpha, maturation of the small pre-existing peroxisome in one large organelle occurs before smaller organelles are split off (Veenhuis et al. 1979). There is probably a large evolutionary difference between these two yeast species, since their codon usages for peroxisomal proteins are nearly opposite to one another (J.M. Goodman, unpublished experiments). Comparison with several other yeasts (Veenhuis et al. 1986, 1987; Zwart et al. 1983) indicates that the pathway described here for C. boidinii is probably the more common one. Peroxisomal proteins generally are homo-oligomeric and often contain cofactors required for enzymatic activity. Alcohol oxidase is typical of a peroxisomal matrix protein; the active enzyme is octameric and contains FAD (Sahm and Wagner, 1973) and possibly other flavins (Sherry and Abeles, 1985). The data shown in Fig. 4 indicate that intraperoxisomal maturation events are not rate-limiting during the course of peroxisomal proliferation, since enzyme activity closely mirrors protein concentration at all time points. This is consistent with the result that alcohol oxidase expressed in H. polymorpha in the absence of methanol induction is capable of acquiring enzymatic activity (Distel et al. 1988). However, there may be species differences in the capacity to mature a given matrix protein; alcohol oxidase from Hansenula protein when expressed in Saccharomyces cerevisiae is incapable of octamerization and binding of cofactor, and therefore inactive, although it can be inefficiently imported into peroxisomes (Distel et al. 1987; van der Klei et al. 1989). While small microbodies are probably the 'organizing center' for peroxisomal proliferation, the data presented here indicate that not all of the small peroxisomes present in cells cultured in glucose undergo proliferation. This behavior could be explained by proposing either that the cell contains two distinct populations of microbodies, only one of which is capable of proliferation, or that there are limiting factors in the cytosol that can promote proliferation of only a fraction of a homogeneous microbi body population. Under some conditions a difference in the ability among peroxisomes to import proteins has been seen in H. polymorpha (Veenhuis et al. 1989). However, there were no apparent differences seen by either histo- or immunoelectron microscopy among unproliferated microbodies in the present study. The function of PMP47 is not known. It is an abundant

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**Fig. 7.** Proliferation of microbodies in Candida boidinii. AO, alcohol oxidase; DHAS, dihydroxyacetone synthase. See Results and Discussion for details.

**Peroxisome proliferation in Candida boidinii**
integral membrane protein of peroxisomes from *C. boidinii* grown on methanol, oleic acid, d-lanine (J. M. Goodman, S. Trapp, H. Hwang, and M. Veenhuis, unpublished data). The primary structure strongly suggests that it spans the bilayer at least twice (McCammon, C. Dowds, C. Slaughter, K. Orth, C. Mounau and J. M. Goodman, unpublished data), consistent with its biochemical characterization (Goodman et al. 1986) There is no clear sequence similarity to the mammalian PMP70 (Kamijo et al. 1990). We have been unable to find a homolog of PMP47 in Saccharomyces cerevisiae using the anti-PMP47 monoclonal antibody, although preliminary experiments using Southern blots indicate that a cross-reacting gene may exist (McCammon, C. Dowds and J.M. Goodman, unpublished data); the existence of a homolog in S. cerevisiae would permit a genetic approach to understanding the function of this protein. Polyclonal antibodies are presently being prepared to continue this examination.

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