The peroxin Pex19p comprising 299 amino acids functions in peroxisomal membrane assembly. We here developed a cell-free system for transport of membrane proteins to peroxisomes. Pex19p interacts with multiple membrane peroxins, including other membrane biogenesis peroxins, Pex16p and Pex26p, involved in matrix protein import. Cell-free synthesized, 35S-labeled Pex19p was targeted to subcellular fractions containing peroxisomes from Chinese hamster ovary-K1 cells as well as peroxisomes isolated from rat liver in an ATP-dependent manner. Such translocation was also reproduced with in vitro synthesized 35S-Pex16p with two transmembrane segments and C-tail anchor-type 35S-Pex26p, upon incubation with 35S-Pex19p in the reaction mixtures containing isolated peroxisomes. The transported 35S-Pex16p and 35S-Pex26p were integrated into membranes as assessed by the sodium carbonate extraction method. Peroxisome-associated and partly Na2CO3-resistant 35S-Pex19p was released to the cytosolic fraction upon incubation in the absence of ATP, whereas 35S-Pex16p and 35S-Pex26p remained in the membranes. Furthermore, not only 35S-Pex19p but also 35S-Pex19p complexes each with 35S-Pex16p and 35S-Pex26p were bound to 35S-Pex3p in vitro. Together, these results strongly suggested that Pex19p translocates the membrane peroxins from the cytosol to peroxisomes in an ATP- and Pex3p-dependent manner and then shuttles back to the cytosol.

Molecular mechanisms of peroxisome biogenesis have been investigated by mostly focusing on the attempt to elucidate how matrix proteins are imported. In contrast, those involving peroxisomal import of membrane proteins and membrane vesicle assembly remain elusive (1–3). In peroxisome-deficient mutant cell lines, including pex3, pex16, and pex19 Chinese hamster ovary (CHO)2 cell mutants and fibroblasts from patients with peroxisome biogenesis disorders, peroxisome membrane assembly is severely impaired; hence, membrane structures, so-called peroxisomal ghosts or membrane remnants, are morphologically and biochemically undetectable (4–10). Peroxisome membrane assembly is initiated upon expression of three peroxins, Pex3p, Pex16p, and Pex19p, in their respective cell mutants. Several distinct steps following the initial stage include import of membrane and matrix proteins, growth, and division or fusion of matured peroxisomes.

We earlier cloned human PEX19 cDNA encoding the 299-amino acid-long peroxin Pex19p with the C-terminal CAAX farnesylation motif by functional complementation strategy using a mutant CHO cell line, ZP119, defective in import of both membrane and matrix proteins (4, 5). Pex19p is localized mostly in the cytosol and only partly associated with peroxisomes (4, 11–14). Pex19p binds to peroxisomal integral membrane proteins (PMPs), including several peroxins such as Pex3p and Pex13p (6, 14–18). Recent studies also reported that Pex19p specifically bound to peroxisome membrane-targeting signal regions of multiple PMPs (10, 19–21) or at regions distinct from the sorting sequences (16). Accordingly, Pex19p has been proposed to function as a membrane-protein chaperone in transporting newly synthesized PMPs from the cytosol to peroxisomes (10, 15–21).

As a further step toward understanding molecular mechanisms underlying the Pex19p functions, we here developed an in vitro assay system for Pex19p targeting to peroxisomes. Moreover, we have provided several lines of evidence that Pex19p interacts with newly synthesized PMPs, directs them to peroxisome membranes, and returns to the cytosol.

**EXPERIMENTAL PROCEDURES**

**Expression and Purification of Recombinant Pex19p**—Expression plasmid encoding human Pex19p fused to glutathione S-transferase (GST-Pex19p) was constructed as follows. PCR was performed using pUCd2Hyg-PEX19 (4) as a template with forward primer PEX19F/BamHI and reverse primer PEX19R/BamHI. A BamHI fragment of PCR products was subcloned into the BamHI site in the pGEX6P-1 vector. GST-Pex19p was expressed in *Escherichia coli* DH5α and purified from cell lysates as described (22). Fusion protein-bound glutathione-Sepharose beads were mixed with 40 units of PreScission protease in cleavage buffer consisting of 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 3 mg/ml pepstatin A, and 1 mM phenylmethylsulfonyl fluoride. The mixture was rotated for 3 h at 4 °C. Pex19p was eluted by centrifugation at 500 × g for 5 min.

**Subcellular Fractionation**—A postnuclear supernatant fraction (PNS) was prepared by centrifugation of the cell homogenate in 0.25 M sucrose/5 mM Hepes-KOH, pH 7.4/0.1% ethanol (homogenizing buffer) from 4 × 107 CHO-K1 cells as described (5, 23). A high speed supernatant (cytosolic fraction) was prepared from PNS by centrifugation at 100,000 × g for 30 min. Rat liver peroxisomes and light mitochondrial fraction were isolated as described (24, 25).

**Cell-free Synthesis of Pex Proteins**—Tandem double hemagglutinin A (HA)2-tagged human PEX19, termed HA2-PEX19, was amplified by PCR using pUCd2Hyg-PEX19 as a template and a respective set of primers, a forward N2F/SalI and a reverse N2R/BamHI. The abbreviations used are: CHOS, Chinese hamster ovary; AOx, acyl-CoA oxidase; EGFP, enhanced green fluorescent protein; PMP, peroxisomal membrane protein; PNS, post-nuclear supernatant; rPex19p, recombinant Pex19p; HA2, tandem double hemagglutinin A.

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precipitated using anti-GFP antibody (Santa Cruz Biotechnology). Immunoprecipitates were analyzed by SDS-PAGE and Fluoro Imaging Analyzer as described above. \(^{35}\)S-HA\(_2\)-Pex19p-bound \(^{35}\)S-FLAG-Pex26p and free \(^{35}\)S-FLAG-Pex26p, if any, were immunoprecipitated using anti-FLAG immunoglobulin G (IgG)-conjugated agarose. Bound proteins were eluted by addition of a large excess of FLAG peptides. The eluate was incubated at 26 °C for 1 h with \(^{35}\)S-FLAG-Pex3p-EGFP. Immunocomplexes of \(^{35}\)S-FLAG-Pex3p-EGFP isolated by immunoprecipitation with anti-GFP antibody were analyzed as above.

Antibodies—Antibodies used for immunoblot were rabbit antibodies to Pex19pN (4), Pex14pC (30), acyl-CoA oxidase (AOX) (23), and HA (29).

Other Methods—Western blot analysis was performed using electrophoretically transferred samples on polyvinylidene difluoride membrane (Bio-Rad) with primary antibodies and a secondary antibody, donkey anti-rabbit IgG antibody conjugated to horseradish peroxidase (Amer sham Biosciences). Antigen-antibody complexes were visualized with ECL-plus Western blotting detection reagent (Amer sham Biosciences) and a Can-Get-Signal Enhancer (Toyobo, Osaka, Japan).

RESULTS

In Vitro Translocation of Pex19p to Peroxisomes—To investigate whether Pex19p targets peroxisome membranes from the cytosol, we attempted to establish a cell-free translocation system of Pex19p. In vitro synthesized \(^{35}\)S-Pex19p was incubated at 26 °C for 1 h in the import assay buffer with PNS fraction from wild-type CHO-K1, pex2 mutant Z65 (31), and pex3 ZPG208 (6) cells and with rat liver peroxisomes. The reaction mixture was separated into organelle and cytosol fractions and analyzed by SDS-PAGE. Pex14p, a peroxisomal membrane protein, was detected in organelle fraction in respective sets of the incubation mixtures, indicating the adequate separation of soluble and organelle fractions (Fig. 1A, left panels). Two types of \(^{35}\)S-Pex19p, authentic and farnesylated forms (4), were detected in both cytosol and organelle fractions (Fig. 1A, lanes 4 and 5), presumably in peroxisomes. To verify this result, we expressed HA\(_2\)-Pex19p in CHO-K1 cells. HA\(_2\)-Pex19p was discernible in particles superimposable on PMP70-stained peroxisomes and in the cytosol (Fig. 1B), thereby suggesting the transport of \(^{35}\)S-HA\(_2\)-Pex19p to peroxisomes. \(^{35}\)S-Pex19p was likewise detected, but in lesser amounts, in membrane fraction when incubated with PNS fraction of peroxisomal membrane remnant-positive pex2 Z65, whereas it was barely associated with the membranes of pex3 ZPG208 defective in peroxisomal membrane biogenesis (lower panels, lanes 4 and 5), consistent with morphological findings. 3 In immunoblot, Pex14p was detected in membrane fraction from both types of CHO cell mutants, consistent with our earlier report (6, 32), suggesting the proper separation in the subcellular fractionation (Fig. 1A, lower panels). \(^{35}\)S-Pex19p was also detectable at a level similar to the result with Z65 in the organelle fraction of other CHO cell mutants defective in matrix protein import, including pex1 ZP107 (33), pex6 ZP164 (5), pex13 ZP128 (34), and pex26 ZP167 (27) (data not shown). \(^{35}\)S-Pex19p associated with the organelles of CHO-K1 was in a significantly higher amount in the assays done in the import assay buffer containing 1 mM ATP and the ATP regeneration system as compared with that incubated in the cell-homogenizing buffer with no addition of ATP (right panel, compare lanes 3 and 5). However, the level of \(^{35}\)S-Pex19p in organelle fraction was ~50% reduced in the presence of 10 mM AMP-PNP in place of ATP (right panel, compare lane 7 with 5). \(^{35}\)S-Pex19p was indeed transported to peroxisomes purified from rat liver, apparently with a much higher...
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![Image](https://example.com/image.png)

**FIGURE 1. ATP-dependent targeting of Pex19p to peroxisomes.** In vitro targeting assays were performed. A, left panel, cell-free synthesized 35S-HA2-Pex19p was incubated at 26 °C for 60 min with PNS fraction from CHO-K1 cells (3 × 105) in homogenizing buffer (lanes 2 and 3), import buffer (lanes 4 and 5), and ATP-depleted import buffer (lanes 6 and 7) or with rat liver peroxisomes (Ps, 6 μg) in import buffer (lanes 8 and 9). PNS fractions from CHO pex2 Z65 and pex3 ZPG208 mutant cells were likewise used for the assay (lower panels in lanes 4 and 5). Reaction mixtures were separated to soluble (S) and organelle (P) fractions and were analyzed by SDS-PAGE. Separation of S and P fractions was assessed by immunoblotting Pex14p, a peroxisomal integral membrane protein. Lane 1, input 35S-Pex19p. 35S-Pex19p was detected by a FLA-5000 imaging analyzer. Solid and open arrowheads indicate farnesylated and authentic 35S-Pex19p, respectively. Right panel, 35S-Pex19p in lanes 3, 5, and 7 from CHO-K1 (upper panel) and that in lane 5 from Z65 and ZPG208 cells (lower panel) were quantitated and represented in an arbitrary unit. B, HA2-tagged Pex19p was expressed in wild-type CHO-K1 cells. Cells were double-stained with antisera to HA (a) and PMP70 (b). Bar. 20 μm. C, upper panel, the reaction mixture containing PNS and 35S-Pex19p in import buffer (lane 7) was separated to cytosolic and organellar fractions (lanes 2 and 3) as in panel A. Organellar fraction was treated with 0.1 M Na2CO3 and separated into soluble (Sol) and integral membrane protein markers, AOx and Pex14p. AOX and Pex14p were detected by immunoblotting. Only AOX B chain is shown. Lower panel, PNS fraction from CHO-K1 cells expressing HA2-Pex19p was likewise separated (lanes 6 and 7). Soluble and membrane fractions (lanes 8 and 9) were isolated by the Na2CO3 treatment of organellar fraction (lane 7). HA2-Pex19p was detected by immunoblotting with anti-HA antibody.

amount of 35S-Pex19p in peroxisome fraction (Fig. 1A, lanes 8 and 9), presumably because ∼50-fold peroxisomes were used as compared with the assay using CHO-K1-derived PNS, based on calculation with catalase-specific activity (23, 35). However, the farnesylated 35S-Pex19p was less detected than that in the assays using PNS fraction in which the farnesylating activity was conceivably higher. Next, 35S-Pex19p in the organellar fraction (Fig. 1C, upper panel, lane 3, equivalent to lane 5 in Fig. 1A, upper panel) was treated with 0.1 M Na2CO3 (28). 35S-Pex19p was partly resistant to the alkaline extraction and recovered in membrane pellet fraction (lane 5), under which membrane and soluble marker proteins of CHO-K1 cells, endogenous Pex14p and AOx, respectively, were adequately separated (lanes 3–5), indicating that membrane-associated 35S-Pex19p partly behaved like an integral membrane protein. Likewise, a part of HA2-Pex19p associated with peroxisomes in CHO-K1 cells shown in Fig. 1B was resistant to alkaline extraction (Fig. 1C, lower panel), hence confirming the finding with 35S-Pex19p in a cell-free system.

To investigate whether Pex19p could be exported from peroxisomes, we resuspended Pex19p-targeted peroxisomes (Fig. 2, upper panel, lane 1) in the homogenizing buffer or the import buffer supplemented with cytosol fraction from CHO-K1 in the absence or presence of AMP-PNP or rPex19p. After a 30-min incubation at 26 °C, the reaction mixtures were separated to organelle and cytosol fractions and analyzed as in Fig. 1A. Pex19p was detected in all of the peroxisome fractions as in the input (upper panel), indicating that peroxisomes in the respective incubation mixtures were sedimented into organelle pellet fractions. 35S-Pex19p in each of the fractions was quantitated (Fig. 2, lower panel). In the homogenizing buffer, 35S-Pex19p was dissociated, up to two thirds, from the organelles into cytosolic fraction (Fig. 2, upper and lower panels, lanes 2 and 3), while about half of the 35S-Pex19p was released in the import buffer containing ATP (lanes 4 and 5). Addition of AMP-PNP instead of ATP apparently reduced the level of 35S-Pex19p in the organelle fraction (lane 7), consistent with the finding in the in vitro targeting assay (see Fig. 1, lane 7), hence implying that a retargeting step...
of 35S-Pex19p was affected. Together, these results suggested that cytosolic Pex19p was targeted to and associated with peroxisomes, translocated back to the cytosol fraction, and retargeted to peroxisomes. Moreover, 35S-Pex19p-associated peroxisomes were likewise incubated in the presence of rPex19p (Fig. 2, upper and lower panels, lanes 8 and 9). Significantly, a reduced amount of 35S-Pex19p was detected in the organelle fraction (compare lanes 5 and 9), with a concomitant increase of 35S-Pex19p in the cytosol fraction (lanes 4 and 8). We interpreted this result to mean that 35S-Pex19p was released from peroxisomes but was not efficiently retargeted to peroxisome membranes because of a larger excess of unlabeled Pex19p, in good agreement with the above observation (lanes 2-7). Moreover, the exogenously added rPex19p was indeed translocated and bound to peroxisome membranes (Fig. 2, upper panel, inset, lanes 8 and 9).

**Pex19p-dependent PMP Import to Peroxisomes and Pex19p Recycling**—We investigated topogenesis of PMPs by taking as a model PMP two integral membrane peroxins, Pex16p with two transmembrane segments (7) and C-tail anchor-type Pex26p (27). In vitro synthesized 35S-Pex16p and 35S-Pex26p were separately incubated with rat liver peroxisomes in the presence or absence of 35S-Pex19p. After centrifugation, peroxisome fractions were analyzed by SDS-PAGE, immunoblot (Pex14p), and Bioimaging Analyzer. Peroxisome fractions (lane 4) were then treated with 0.1 M Na2CO3 and separated into soluble (Sol.) and organelle membrane (Memb.) fractions (lanes 5 and 6). Both fractions were assessed by immunoblot of Pex14p and AOx-B. Lanes 1 and 2, input. Right, 35S-Pex16p and 35S-Pex26p bands (lanes 3 and 4) were quantitated as in Fig. 2 and were represented by taking the amount in lane 3 as 1. Shaded arrowhead indicates 35S-FLAG-Pex16p (upper panel) and 35S-FLAG-Pex26p (lower). 8, release of Pex19p from organelles. Left, PMP import was done using 35S-Pex16p (upper panel) and 35S-Pex26p (lower panel) with 35S-Pex19p as in panel A. Peroxisome fractions (lane 1) were resuspended in the import buffer containing CHO-K1-derived cytosol and were incubated for 30 min at 26 °C with or without addition of rPex19p (40 μg/ml). The reaction mixtures were separated into cytosol (S) and organelle (P) fractions (lanes 2-5). Peroxisomes in the input and S and P fractions were assessed as in panel A. Right, both farnesylated and authentic forms of 35S-Pex19p, 35S-Pex16p, and 35S-Pex26p in each fraction were quantitated as in Fig. 1 and, respectively, represented in an arbitrary unit. Note that 35S-Pex19p was released to the cytosol fraction.
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**FIGURE 4. Cargo-loaded Pex19p binds to Pex3p.** A. 35S-FLAG-Pex16p and 35S-HA2-Pex19p were separately translated and incubated at 4 °C for 2 h with 35S-FLAG-Pex3p-EGFP as indicated at the top. Immunoprecipitation was performed using anti-GFP antibody in the absence (lane 4) and presence (lanes 5 and 6) of 35S-FLAG-Pex3p-EGFP, and the immunocomplexes were analyzed by SDS-PAGE and Bioimaging Analyzer. Lanes 1–3, 10% input (0.5 μl); lanes 4–6, immunoprecipitates. Arrow indicates 35S-FLAG-Pex3p-EGFP; open, solid, and shaded arrowheads show 35S-HA2-Pex19p, farnesylated 35S-HA2-Pex19p, and 35S-FLAG-Pex16p, respectively. Molecular markers are on the right. Note that 35S-HA2-Pex19p with a calculated mass of 37 kDa and its farnesylated form showed an aberrant mobility as noted for Pex19p (4). B, essentially the same assays were performed as in panel A except for using 35S-FLAG-Pex26p. Shaded arrowhead indicates 35S-FLAG-Pex26p. C. 35S-FLAG-Pex26p and 35S-HA2-Pex19p were separately translated in vitro, mixed, and incubated at 4 °C for 60 min. The reaction mixture was subjected to immunoprecipitation using anti-FLAG antibody-conjugated agarose. Bound proteins were eluted with excess FLAG peptides. The resulting eluate (lane 3) and 35S-HA2-Pex19p (lane 2) were incubated with 35S-FLAG-Pex3p-EGFP as indicated at the top. 35S-FLAG-Pex3p-EGFP was immunoprecipitated and analyzed as in panel A. Lane 1, 10% input (0.5 μl); lanes 2 and 3, 50% input; lanes 4–6, immunoprecipitates. Shaded arrowhead indicates 35S-FLAG-Pex26p. Dot, apparently degraded products as seen in the input (lanes 2 and 3).

for membrane and soluble proteins of peroxisomes, were detected in the membrane and soluble fractions, respectively (Fig. 3A, upper and lower left panels, lanes 5 and 6), indicating that 35S-Pex16p and 35S-Pex26p were integrated to peroxisome membranes. Interestingly, about half or less of 35S-Pex19p was also resistant to the alkaline extraction (lanes 5 and 6), implying its apparently membrane-integrated form. Next, 35S-Pex16p- and 35S-Pex19p-targeted peroxisomes (Fig. 3B, upper panel, lane 1) were resuspended in the import assay buffer containing CHO-K1-derived cytosol and were incubated at 26 °C for 30 min as in Fig. 2. The input peroxisomes were likewise recovered in organelle pellet fractions as verified by Pex14p immunoblot (Fig. 3B). About one half of 35S-Pex19p was released from peroxisomes, whereas 35S-Pex16p mostly remained in peroxisome membranes (Fig. 3B, upper left panel, lanes 2 and 3). Addition of a larger excess of rPex19p to the reaction mixture significantly reduced the amount of 35S-Pex19p associated with peroxisomes, with a concomitant 2-fold increase of 35S-Pex19p in the soluble fraction, under which the level of 35S-Pex16p in the organelle and soluble fractions was little affected (upper left panel, lanes 4 and 5; upper right panel). Similar results were obtained with 35S-Pex26p, where the decrease of organelle-associated 35S-Pex19p and increase of 35S-Pex19p in the soluble fraction (Fig. 3B, lower right panel, lanes 1–5; lower right panel) were distinct. Taken together, these results strongly suggested that PMPs such as the membrane peroxins of two different types are recruited by Pex19p in the cytosol, transported to peroxisomes, and integrated into peroxisome membranes. Upon or after integration of PMPs, Pex19p is likely released to the cytosol and recycled for another round of recruiting and targeting of PMPs.

**Pex19p Recruits PMPs to Pex3p**—A potential docking receptor on peroxisomes for Pex19p was very recently suggested to be Pex3p (36). Therefore, we investigated whether ternary complexes of Pex3p-Pex19p-PMP cargo are formed. 35S-HA2-Pex19p, 35S-FLAG-Pex16p, and 35S-FLAG-Pex3p-EGFP were synthesized by耦合 transcription and translation, 35S-FLAG-Pex16p and 35S-HA2-Pex19p were incubated for 2 h with 35S-FLAG-Pex3p-EGFP. 35S-FLAG-Pex3p-EGFP was immunoprecipitated with anti-GFP antibody, and the immunocomplexes were analyzed. 35S-HA2-Pex19p and 35S-FLAG-Pex16p were detected specifically in the immunocomplexes of 35S-FLAG-Pex3p-EGFP (Fig. 4A, lanes 1–4 and 6), indicating that these three peroxins formed a ternary complex. In contrast, 35S-FLAG-Pex16p was not detectable in the 35S-FLAG-Pex3p-EGFP immunoprecipitates when only these two were incubated (lane 5). Essentially the same results were obtained in the ternary complex formation assay using 35S-HA2-Pex19p, 35S-FLAG-Pex26p, and 35S-FLAG-Pex3p-EGFP (Fig. 4B). Next, 35S-FLAG-Pex26p-loaded 35S-HA2-Pex19p was prepared by in vitro co-translation followed by immunoprecipitation with anti-FLAG IgG-agarose (Fig. 4C). 35S-HA2-Pex19p-bound 35S-FLAG-Pex26p fraction, including free 35S-FLAG-Pex26p if any, that had been eluted with FLAG peptides was incubated with 35S-FLAG-Pex3p-EGFP (lanes 1–3). Immunoprecipitation of 35S-FLAG-Pex3p-EGFP gave rise to a complex comprising 35S-FLAG-Pex3p-EGFP, 35S-HA2-Pex19p, and 35S-FLAG-Pex26p (Fig. 4C, lanes 4 and 6), thereby demonstrating that these three formed a ternary complex. Cargo-free 35S-HA2-Pex19p likewise bound to 35S-FLAG-Pex3p-EGFP (lane 5). Together with the results shown in Fig. 3, we interpreted these findings to mean that Pex19p transport PMP cargoes to peroxisomes, most likely by direct docking to Pex3p, consistent with the findings in vivo (36).

**DISCUSSION**

In earlier studies by several groups including ours (4, 12, 13), Pex19p was shown to be essential at early steps of peroxisomal membrane biogenesis such as that involving the recognition of multiple PMPs (14, 16–18, 20). Defect of Pex19p function indeed impairs peroxisomal mem-

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4 Y. Matuzono, and Y. Fujiki, to be reported elsewhere.
brane assembly occurring prior to the import of peroxisomal matrix enzymes in yeast (12, 13) and mammalian cells such as CHO cells and human fibroblasts (4, 5, 14).

In the present work, we established a cell-free Pex19p translocation system. Moreover, in this system, PMPs including two membrane-spanning Pex16p and a C-tail-anchored-type Pex26p were transported in a Pex19p-dependent manner and integrated to peroxisome membranes. Furthermore, Pex19p complexes with PMPs such as Pex16p and Pex26p bound to Pex3p in vitro, indicating that the ternary complexes were formed. The profiles of targeting to peroxisomes of Pex19p and its truncated variants were superimposable to those of their binding to Pex3p, as assessed by co-immunoprecipitation and/or yeast two-hybrid assays. By taking this observation into consideration, we interpreted the findings on the ternary complex formation to mean that Pex19p-PMP complexes were transported to their docking site Pex3p on peroxisomes, in good agreement with the recent suggestion that Pex3p is required for Pex19p docking to peroxisomes (36). With regard to the physiological role, Pex19p has been thought to function as a PMP chaperone (17, 37) and an import receptor that mediates the transport of PMPs to peroxisomes (15, 38) by interacting with their peroxisome membrane-targeting signal (19, 20, 39), except for Pex3p (36). Fang et al. (36) recently reported that Pex3p was required for recruiting and docking of Pex19p-PMP complexes to peroxisomes. Our findings in the present work favor such models.

The translocation to peroxisomes of Pex19p requires ATP and is affected by AMP-PNP. Whether any factor is involved in the hydrolysis or binding of ATP remains to be defined. Meanwhile, the AAA ATPase family peroxins, Pex1p and Pex6p, essential for the import of matrix enzymes in yeast (12, 13) and mammalian cells such as CHO cells and human fibroblasts (4, 5, 14).

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