Dual function of Sec16B

Endoplasmic reticulum-derived protein secretion and peroxisome biogenesis in mammalian cells

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The origin of peroxisomes has long been disputed. However, recent evidence suggests that peroxisomes can be formed de novo from the endoplasmic reticulum (ER) in yeast and higher eukaryotes. Sec16A and Sec16B, mammalian orthologs of yeast Sec16, are scaffold proteins that organize ER exit sites by interacting with COPII components. We recently demonstrated that Sec16B, but not Sec16A, regulates the transport of peroxisomal biogenesis factors from the ER to peroxisomes in mammalian cells. The C-terminal region of Sec16B, which is not conserved in Sec16A, is required for this function. The data suggest that Sec16B in ER areas other than ER exit sites plays this role. Our findings provide an unexpected connection between at least part of the COPII machinery and the formation of pre-peroxisomal vesicles at the ER, and offer an explanation of how secretory and peroxisomal trafficking from the ER are distinguished.

Peroxisomes are Formed de novo from the ER

Eukaryotic cells have various organelles whose functions are achieved through proteins and enzymes that are uniquely localized in organelles. As most proteins are encoded by nuclear DNA, organelle proteins synthesized on ribosomes must be faithfully transported to their working places. To meet this requirement, cells employ two mechanisms: post-translational translocation and vesicular transport. In general, autonomous organelles,

which multiply through growth and division, utilize the post-translational pathway and organelles in the secretory and endocytic pathways use the vesicular transport system. The ER is the starting place for the secretory pathway, where newly synthesized proteins are incorporated into COPII vesicles that are destined for the Golgi apparatus.

For a long time, peroxisomes were acknowledged as being autonomous organelles that import soluble enzymes and also peroxisome membrane proteins (PMPs) via the post-translational translocation pathway. However, recent studies provided compelling evidence of the involvement of the ER in de novo peroxisome biogenesis (reviewed in refs. 1–3). In 2005, Tabak and colleagues demonstrated that, in yeast, a peroxisomal biogenesis factor, Pex3, originates in the ER and then moves to peroxisomes in a manner dependent on a cytosolic peroxisomal biogenesis factor, Pex19.⁴ Pex3 is not the sole example of a PMP traveling via the ER. A recent study showed that a comprehensive set of 16 PMPs in yeast are transported through this pathway.⁵ In mammals, Pex16, another peroxisomal biogenesis factor, also moves from the ER to peroxisomes in a Pex19-dependent manner.⁶ *Arabidopsis thaliana* Pex16 (AtPex16) was also demonstrated to be delivered via the ER.⁷ Very recent reconstitution studies involving yeasts *Saccharomyces cerevisiae* and *Pichia pastoris* revealed that vesicle carriers containing PMPs are formed from the ER in an ATP, cytosol and Pex19-dependent manner.^{8,9}

Key words: Sec16B, peroxisomes, endoplasmic reticulum, vesicular transport, Pex3, Pex16

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Figure 1. Model of the role of Sec16B in the formation of membrane carriers destined for peroxisomes. As in the case of Sec16 in ER exit sites, Sec16B may interact with vesicle coat components that facilitate the deformation of ER membranes, and the incorporation of PMPs such as Pex16 and Pex3 into nascent vesicles. Alternatively, Sec16B may function as a scaffold that interacts directly or indirectly with PMPs and putative fission factors on ER membranes. In the absence of Sec16B, Pex3 seems to be degraded by the ubiquitin-proteasome system. In yeast, transport carriers are detached from ER membranes at budding sites,^{8,9} but in mammalian cells, pre-peroxisomal structures (subdomains in the smooth ER) may be associated with the peroxisomal reticulum.²⁸

Identification of Mammalian Sec16B

Sec16 was first characterized in *S. cerevisiae* as a 240-kDa peripheral membrane protein that interacts with COPII coat components and facilitates vesicle budding, possibly by preventing premature coat disassembly.^{10–12} In *P. pastoris* and metazoans, COPII assembly occurs at one of the ER subdomains, termed ER exit sites or the transitional ER. In *P. pastoris*, Sec16 defines ER exit sites perhaps by linking COPII subunits on nascent vesicles through its self-association capability.¹³ Mammalian orthologs of Sec16 were not discovered until recently, three groups independently identifying them in the mid-2000s. In 1999, we isolated two mammalian proteins (p250 and p125/Sec23ip) that interact with a COPII component, Sec23,¹⁴ and subsequently showed that p250 is a mammalian Sec16 ortholog (Sec16A; 2,357 amino acids).¹⁵ Knockdown experiments demonstrated that Sec16A plays roles in the organization of ER exit sites and in protein export from the ER. Its activity appears to be regulated through phosphorylation by ERK2.¹⁶ Using the central region of Sec16 conserved in *S. cerevisiae* and *P. pastoris* as a probe, David Stephens and colleagues searched the genome sequence and identified a human Sec16 ortholog (Sec16A).¹⁷

They demonstrated by immunoelectron microscopy that Sec16A is localized in cup-like structures of ER exit sites, which are close but discrete from the location of COPII components.^{18,19} Ben Glick's group also used a homology search approach and identified Sec16B (117 kDa; 1,061 amino acids) as well as Sec16A.²⁰ Sec16A appears to be the primary Sec16 ortholog because of the similarity in molecular mass. Like Sec16A, Sec16B is localized to ER exit sites and participates in protein secretion. An immunoprecipitation experiment showed that Sec16A and Sec16B, when ectopically expressed in cells, form heteromeric complexes. Sec16B was originally described as a regucalcin gene promoter region-related protein (RGPR-p117).²¹ The role of Sec16B/RGPR-p117 as a transcription factor needs to be confirmed because of the lack of evidence of its direct binding to the regucalcin gene promoter region.

Sec16B Participates in Peroxisome Biogenesis

We recently demonstrated that Sec16B plays a pivotal role in peroxisome biogenesis in mammalian cells.²² This discovery began with the finding that depletion of Sec16B by RNAi not only disrupted ER exit sites but also caused the elongation of peroxisomes. This accidental finding encouraged us to scrutinize the role

of Sec16B in the context of peroxisome biogenesis. We focused on two peroxisomal biogenesis factors, Pex3 and Pex16, because previous studies suggested that these two integral membrane proteins are involved in de novo formation of peroxisomes from the ER.^{1–3} In the post-translational translocation reaction, the former functions as a PMP import receptor and the latter as a Pex3-Pex19 receptor.²³ When overexpressed, Sec16B was targeted to the entire ER accompanied by dramatic redistribution of Pex3 and Pex16 from peroxisomes to the ER, and a substantial loss of peroxisomes. Knockdown of Sec16B also caused redistribution of Pex16 to the ER and reduced the number of peroxisomes concomitantly with their elongation. In the case of Pex3, depletion of Sec16B induced its degradation by the ubiquitin-proteasome system. This Sec16B-deficient phenotype was not observed in Sec16A-deficient cells, suggesting different roles of the two Sec16 paralogs. Rescue experiments demonstrated that an N-terminally truncated mutant (amino acids 272–1,061) compensated for the loss of Sec16B. This mutant was not localized properly because it lacks a positively charged region on the N-terminal side of the central conserved domain, which is required for the targeting of Sec16 to ER exit sites.^{18,24} In contrast, a C-terminally truncated fragment (amino acids 1–713) was found to be localized in ER exit sites, but did not reverse the Sec16B depletion effect on peroxisome biogenesis. These results raise the possibility that ER exit sites are not responsible for peroxisome biogenesis and suggest that the C-terminal 348 amino acids are important for the function of Sec16B in peroxisome biogenesis. This region is not conserved in Sec16A and is characterized by a unique amino acid composition rich in Gly (12.6%) and Ser (13.5%).

Remaining Questions

How does Sec16B regulate the transport of Pex3 and Pex16 from the ER to peroxisomes? Given that Sec16 interacts with COPII coat components,¹¹ it is tempting to speculate that Sec16B supports the assembly of coat components for vesicle carriers destined for peroxisomes by

directly interacting with the coat (Fig. 1). At present, coat components for ER-to-peroxisome carriers are not known. The coat for the carriers is likely not COPII because inhibitors of COPII do not block Pex3-mediated peroxisomes biogenesis in vivo^{25,26} or Pex19-dependent in vitro vesicle formation.⁸ This idea is consistent with the fact that Sec16B lacking the ER exit site-targeting signal is functional as to ER export of Pex16. To identify the coat, in vitro vesicle formation assay may be a powerful approach.^{8,9} Another possible function of Sec16B is to serve as a scaffold for the assembly of ER-embedded biogenesis factors such as Pex3 and Pex16 and to recruit biogenesis factors such as Pex19 and putative fission factors from the cytosol. Ma et al. have proposed that Pex19 acts as a chaperone for the assembly of PMPs on the ER membrane after protein insertion. Regardless of which possibility is correct, it is important to identify Sec16B-binding proteins other than COPII components.

Another important question is where on the ER membrane de novo peroxisome formation occurs. Studies on *S. cerevisiae* demonstrated that Pex3 initiates the formation of a pre-peroxisome compartment from a subdomain of the ER.²⁷ In mouse dendritic cells, Pex13 and PMP70 were found to be present in specialized subdomains of the smooth ER that are connected with the peroxisomal reticulum from which mature peroxisomes arise.²⁸ The latter may suggest that, in mammalian cells, the region for de novo peroxisome formation is a contact site between the smooth ER and peroxisomes. If this were the case, vesicle formation would not be required, and coat components might only concentrate peroxisomal biogenesis factors with the fission machinery at the contact site. Searching for approaches to block the fission of pre-peroxisome compartments or the movement of Pex16 from the contact site to peroxisomes may increase our understanding of the mechanism by which peroxisomal membrane proteins leave the ER.

Another intriguing question is whether or not the role of Sec16 as a peroxisomal biogenesis factor has been conserved throughout evolution. Mammals, frogs, and perhaps zebrafish have two Sec16 paralogs, whereas flies, *Caenorhabditis*

elegans and yeasts have only one Sec16 species. Although *A. thaliana* appears to have two genes, they are aligned in tandem on chromosome 5 and are essentially the same. One possibility is that Sec16B acquired a function as a peroxisomal biogenesis factor during evolution, concomitantly with a modest loss of its secretion function. Depletion of Sec16B, compared with Sec16A depletion, had a lesser effect on protein export from the ER through the secretory pathway. Thus, in organisms having only one Sec16 species, Sec16 may not participate in peroxisome biogenesis. It should be noted that peroxisomal biogenesis factors are not well conserved.² In *S. cerevisiae*, Pex16 appears to be missing, and *Y. lipolytica* Pex16 is more likely a negative regulator of peroxisome fission. As to Pex3, its topology differs with the species. An alternative possibility is that the single Sec16 present in yeast and other organisms has a dual function in secretion and peroxisome biogenesis. This question will be answered soon.

Since 2009, several studies revealed that a polymorphism of the Sec16B gene is associated with obesity (for example, see ref. 29). There is so far no experimental evidence of a defect or perturbation of peroxisome function associated with this polymorphism. However, as the β -oxidation of fatty acids occurs in peroxisomes, changes in the expression level and/or function of Sec16B are somehow related to the onset of obesity. In this context, it is important to examine the correlation of Sec16B gene polymorphism with peroxisome capacity and lipid content of adipocytes.

The relationship, if any, between ER-peroxisome and ER-Golgi transport remains to be clarified. A recent study revealed that ER-Golgi transport and ER-peroxisome transport in *S. cerevisiae* are mediated by a common set of proteins (Sec20, Sec39 and Dsl1).³⁰ These proteins are subunits of the yeast Ufe1 complex, a large complex on the ER that mediates the tethering and fusion of Golgi-derived COPI vesicles with the ER membrane.³¹ Because the Ufe1 complex is principally localized in the ER, its possible primary role is to accept membranes from peroxisomes (retrograde transport), and the apparent arrest in transport from the ER to peroxisomes may be an indirect

consequence of impaired retrograde transport. Because anterograde and retrograde membrane flow are closely coupled, a rapid effect on retrograde transport leads to a block in anterograde transport, as observed for the transport between the ER and the Golgi apparatus.³² Some evidence suggests the presence of the retrograde pathway from peroxisomes.³ It is possible that Arf1 and COPI, the coat for vesicles involved in retrograde transport from the Golgi to the ER, participate in this pathway,³³ although some studies showed that COPI is not required for peroxisome biogenesis.^{25,26} An alternative possibility is that yeast Sec20, Sec39 and Dsl1 may directly participate in anterograde transport to peroxisomes. There are several lines of evidence implying that the Ufe1 complex (the syntaxin 18 complex in higher eukaryotes³⁴⁻³⁶) participates in anterograde transport from the ER in higher eukaryotes. *A. thaliana* Maigo2, the ortholog of yeast Tip20 in the Ufe1 complex, participates in the transport of storage protein precursors from the ER to the Golgi apparatus.³⁷ In the case of mammalian cells, ZW10, the ortholog of yeast Dsl1, is known to interact with microtubule motor dynein-dynactin.³⁸ This motor protein has been reported to be required in the early stage of peroxisome biogenesis.³⁹

Our work may explain how secretory and peroxisomal traffic from the ER are distinguished. Given that Sec16B, when expressed at reasonable levels, shows a tight ER exit site localization indistinguishable from that of Sec16A, it is somewhat surprising that it participates in peroxisome biogenesis. Detailed characterization of Sec16B may offer further insights into the mechanism underlying the de novo formation of peroxisomes from the ER.

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