Review

Lipids and lipid domains in the peroxisomal membrane of the yeast *Yarrowia lipolytica*

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Received 25 May 2006; received in revised form 10 August 2006; accepted 18 August 2006

Abstract

Biological membranes have unique and highly diverse compositions of their lipid constituents. At present, we have only partial understanding of how membrane lipids and lipid domains regulate the structural integrity and functionality of cellular organelles, maintain the unique molecular composition of each organelar membrane by orchestrating the intracellular trafficking of membrane-bound proteins and lipids, and control the steady-state levels of numerous signaling molecules generated in biological membranes. Similar to other organelar membranes, a single lipid bilayer enclosing the peroxisome, an organelle known for its essential role in lipid metabolism, has a unique lipid composition and organizes some of its lipid and protein components into distinctive assemblies. This review highlights recent advances in our knowledge of how lipids and lipid domains of the peroxisomal membrane regulate the processes of peroxisome assembly and maintenance in the yeast *Yarrowia lipolytica*. We critically evaluate the molecular mechanisms through which lipid constituents of the peroxisomal membrane control these multistep processes and outline directions for future research in this field.

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Keywords: Organelle biogenesis; Membrane structure; Membrane domain; Membrane lipid; Lipid raft; Peroxisome assembly

1. Introduction

Recent advances in our understanding of the strategies and molecular mechanisms that evolutionarily diverse organisms have evolved for assembling, maintaining, propagating and inheriting the peroxisome, an organelle known for its essential role in lipid metabolism, marked a Renaissance period in the field of peroxisome biogenesis [1]. One of the hallmarks of this period is the evolution of our view on the peroxisome as a subcellular compartment that originates from the endoplasmic reticulum (ER) [1–9]. Moreover, it appears that, analogous to some organelles of the secretory endomembrane system, peroxisomes found in the yeast *Y. lipolytica* and in mouse dendritic cells constitute a multi-compartmental endomembrane system [1,5,6,10–13]. This peroxisomal endomembrane system exists as a dynamic organelle population consisting of several structurally distinct compartments that differ in their import competency for various proteins [1,5,6,10–13]. It seems that the individual compartments of the peroxisomal endomembrane system found in *Y. lipolytica* and mouse dendritic cells undergo a multi-step conversion to mature peroxisomes in a time-ordered manner [1,5,6,10,11]. Studies in *Y. lipolytica* [1,5,10] and mouse dendritic cells [1,6,11] have suggested two models for the multistep process of peroxisome assembly and maturation. In both models, the targeting of peroxisomal membrane proteins to the membrane of the early intermediates in a pathway precedes, and is mandatory for, the import of soluble peroxisomal proteins into the matrix of later intermediates [1,5,6,10,11]. A crucial evaluation of the two peroxisome assembly and maturation pathways proposed for *Y. lipolytica* and mouse dendritic cells will require their testing in other organisms and cell types. Such studies might include the purification and characterization of different peroxisomal subforms that have been found in various yeast species and mammalian cells [14–20] and the analysis of the spatiotemporal dynamics of peroxisome protein localization to distinct peroxisomal populations in living cells.
Despite the substantial progress achieved recently in developing a new paradigm of peroxisome biogenesis [1,4–13], several fundamental issues remain unresolved. The scope of this review article is to summarize the growing evidence in support of the essential role that individual lipid species and lipid domains in the peroxisomal membrane of the yeast *Y. lipolytica* play at the checkpoints of the multistep processes of peroxisome assembly, maintenance and propagation. In addition, we outline the most important unanswered questions related to the molecular mechanisms through which membrane lipids regulate the assembly, remodeling and functioning of protein complexes in the peroxisomal membrane at each of these checkpoints. We provide suggestions for future research in this vibrant and rapidly evolving field.

2. Exit of pre-peroxisomal carriers from the ER is the initial step of lipid sorting to the peroxisomal membrane

A growing body of evidence supports the view that the peroxisomal endomembrane system in evolutionarily diverse organisms originates from the ER [1,5,6,10,11,21–28]. The dynamic flow of membrane-enclosed carriers through this endomembrane system is initiated by the sorting of a limited subset of peroxisomal membrane proteins (PMPs), referred to as group I PMPs [5], to the ER. At least three group I PMPs in the yeasts *Saccharomyces cerevisiae*, *Yarrowia lipolytica* and *Hansenula polymorpha*, including Pex2p, Pex3p and Pex16p, are initially targeted to the perinuclear and cortical ER also known as “general” ER [22,24,27]. In *S. cerevisiae*, the group I PMP Pex3p then moves laterally from the “general ER” to a distinct domain of the ER, thereby creating a template for the budding of small pre-peroxisomal vesicles [24]. The term pre-peroxisomal template was coined for this specialized domain of the ER membrane in yeasts [5]. The vesicular pre-peroxisomal carriers that bud from the pre-peroxisomal template in *Y. lipolytica* carry group I PMPs but lack secretory and ER resident membrane proteins [21,22]. In addition to group I PMPs, the membrane of these ER-derived carriers incorporates a portion of lipids that are synthesized in the ER membrane [21,22,24,26,27]. Thus, the initial step of lipid sorting to the peroxisomal membrane in yeast cells occurs during exit of pre-peroxisomal carriers from the ER.

What could be the mechanism for the observed lateral segregation of group I PMPs from secretory and ER resident membrane proteins within the ER membrane of yeast cells? What role, if any, could individual lipid species and lipid domains of the ER membrane play in this process? Some recent findings suggest that ergosterol- and ceramide-rich (ECR) domains in the ER membrane of yeast can function as sorting platforms for segregating ER resident proteins, at least two groups of secretory and plasma membrane proteins, and group I PMPs from each other. In fact, glycosylphosphatidylinositol (GPI)-anchored proteins in *S. cerevisiae* cells exit the ER in vesicles that are distinct from those that carry many other secretory and plasma membrane proteins that are not anchored to GPI [29]. Analysis of specific requirements for the packaging of GPI-anchored proteins into ER-derived vesicles suggested that their partitioning into ceramide-rich lipid raft domains, which are clustered in distinct regions of the ER, is responsible for their lateral segregation from non-GPI-anchored secretory and plasma membrane proteins [29–35]. In yeasts, these detergent-resistant membrane domains are formed in the ER [36]. Importantly, the membrane of the ER-derived pre-peroxisomal vesicles in the yeast *Y. lipolytica* has unusual ECR domains that resist solubilization by cold nonionic detergents and are similar to detergent-resistant lipid raft domains found in the membrane of *S. cerevisiae* ER [37]. Therefore, it is tempting to speculate that the ECR domains in the membrane of *Y. lipolytica* serve as a sorting station for segregating group I PMPs from secretory and ER resident membrane proteins. It is conceivable that the availability of a yeast-based *in vitro* assay for the budding of distinct populations of vesicles from the donor ER membrane [29–33] could help to unravel the mechanism by which these lipid domains govern the sorting of membrane lipids and proteins within the ER membrane, thereby promoting their selective packaging into pre-peroxisomal and secretory vesicles.

3. Dynamic lipid domains in the membranes of immature peroxisomal vesicles are essential for the initial step of their multistep conversion to mature peroxisomes

In the yeast *Y. lipolytica*, the population of peroxisomes in a cell consists of six distinct vesicular subforms that have been purified and characterized [38]. The six peroxisomal subforms are related through the ordered conversion of one subform to another, being organized into a multistep peroxisome assembly pathway [5,10,38,39]. The pathway operating in *Y. lipolytica* leads to the formation of mature peroxisomes, P6, carrying the complete set of matrix and membrane proteins and membrane lipids. This pathway operates by conversion of five immature peroxisomal vesicles, termed P1 to P5, to mature peroxisomes in a temporally ordered manner from P1 to P6 [38,39]. The earliest intermediates in the pathway, the ER-derived immature peroxisomal vesicles P1 and P2, contain most of PMPs associated with mature peroxisomes but carry only a few matrix proteins [38,39]. An initial step in the multistep peroxisome assembly pathway acting in *Y. lipolytica* cells involves the fusion of P1 and P2 to yield larger vesicles, P3 [38,40]. Fusion between P1 and P2 has been reconstituted *in vitro* [38]. It is driven by ATP hydrolysis, requires cytosolic proteins and depends on the peroxins Pex1p and Pex6p [38], two AAA ATPases essential for peroxisome biogenesis [12,41,42]. Fusion of P1 and P2 is a multistep process that includes priming, docking and fusion events [40].

Our recent findings in *Y. lipolytica* provided evidence that membrane bilayers of both P1 and P2 exist in two lipid phases [37]. A detergent-soluble phase is enriched in glycerophospholipids but has only minute amounts of ergosterol and ceramide. The other phase resists solubilization by cold nonionic detergents and is highly enriched in ergosterol and ceramide. The term ergosterol- and ceramide-rich (ECR) domains was coined for this distinct phase of the peroxisomal membrane bilayer [37]. ECR domains in the membranes of P1 and P2
vesicles contain only minor portions of various glycerophospholipids. Similar to the well-characterized lipid raft domains in the plasma membranes of evolutionarily diverse eukaryotic organisms [43], ECR domains of unprimed P1 and P2 in *Y. lipolytica* cells represent a substantial fraction of their membrane bilayers, with about half of membrane lipids and proteins being associated with these membrane domains [37]. Furthermore, both ECR domains in the membranes of *Y. lipolytica* P1 and P2 and lipid raft domains in the plasma membranes of various eukaryotic cells are extremely dynamic [37,43,44]. When P1 and P2 vesicles are stimulated for priming and docking, numerous protein constituents of ECR domains rapidly move from these domains to an ergosterol- and ceramide-poor portion of the membrane [37]. Similarly, lipid raft proteins in the plasma membrane are extremely mobile and undergo rapid lateral diffusion in the membrane bilayer [44]. On the other hand, some key properties of ECR domains in the peroxisomal membrane bilayer of *Y. lipolytica* clearly distinguish them from lipid raft domains in the plasma membrane [37]. First, ceramide is the only sphingolipid component of ECR domains. The sphingosine base of this sphingolipid lacks a polar head group [45]. In contrast, sphingolipids of lipid rafts in the plasma membrane have large polar head groups attached to their sphingosine base [45]. Therefore, ceramide in model membrane bilayers forms detergent-insoluble lipid domains that are significantly more stable than those formed in the presence of plasma membrane sphingolipids [46]. Interestingly, this characteristic feature of ceramide is responsible for its ability to stabilize lipid raft domains in the ER membrane of the yeast *S. cerevisiae*, thereby creating sorting platforms for segregating ER resident proteins, at least two groups of secretory and plasma membrane proteins, and group I PMPs from each other [29–35] (see above). Second, ceramide, which spontaneously flips across the membrane bilayer with a half-time of ~10 min [45], in ECR domains of the membranes of P1 and P2 is distributed symmetrically between the two leaflets of the bilayers [37]. This is in contrast to asymmetric distribution of sphingolipids, the abundant constituents of lipid rafts, across the lipid bilayer of the plasma membrane. Because, in contrast to ceramide, sphingolipids are unable to move across the bilayer [45], they are restricted to lipid raft domains in the outer leaflet of the plasma membrane [47,48]. It remains to be seen whether the symmetric distribution of ceramide across the peroxisomal membrane in *Y. lipolytica* and its ability to flip between the two leaflets of the bilayer promote the coordination of events that occur in the cytosolic and luminal leaflets of ECR domains.

In *Y. lipolytica*, ECR domains in the membranes of P1 and P2 are dynamic assemblies of a distinct set of lipids and proteins, including Pex1p, Pex6p, GTP-binding and hydrolyzing proteins (GTP-bp), and proteins that specifically bind to phosphatidylinositol-4-phosphate [PI(4)P] and phosphatidylinositol-4,5-bisphosphate [PI(4,5)P₂] (PI(4)P-bp and PI(4,5)P₂-bp, respectively) [37]. Our recent findings provided evidence that these membrane domains in *Y. lipolytica* function as an organizing platform for the fusion of P1 and P2 by orchestrating the spatial and temporal reorganization of a protein team that only transiently resides in ECR domains and controls peroxisome fusion [37]. Based on these findings, we suggested the following model for the multistep remodeling of the peroxisome fusion machinery in the membrane bilayers of P1 and P2 vesicles in *Y. lipolytica* (Fig. 1[37]). In unprimed P1 and P2, all identified essential components of this machinery, including Pex1p, Pex6p, GTP-bp, PI(4)P-bp and PI(4,5)P₂-bp, are attached to the cytosolic face of ECR domains. The lateral movement of P1-bound Pex1p and P2-associated Pex6p from ECR domains to an ergosterol- and ceramide-poor portion of the membrane initiates priming of both fusion partners. This essential event in the process of activating P1 and P2 for their subsequent docking includes at least three consecutive steps. The initial ergosterol-dependent step is followed by a PI(4)P-requiring step, which precedes a step that needs PI(4,5)P₂. After being segregated from ECR domains, both P1-bound Pex1p and P2-associated Pex6p are released to the cytosol. Such release of both AAA ATPases from ergosterol- and ceramide-poor portions of the membranes of both fusion partners is mandatory for their priming. The release of these AAA ATPases to the cytosol occurs in two steps. The first step depends on cytosolic proteins, whereas the next step is promoted by ATP hydrolysis. Primed peroxisomal vesicles then undergo docking. Docking of primed P1 and P2 is a multistep process. It begins with the lateral movement of PI(4,5)P₂-bp in the membranes of P1 and P2 from ECR domains to ergosterol- and ceramide-poor portions of their membranes. This lateral movement of PI(4,5)P₂-bp occurs in three consecutive steps. The first step depends on ergosterol in the membrane bilayers of both fusion partners. The second step needs Pex1p that resides in ECR domains of P2 vesicles. The third step requires GTP hydrolysis by GTPase(s), perhaps by GTP-bp in ECR domains of P1 vesicles. The docking-specific sliding of PI(4,5)P₂-bp in the membranes of P1 and P2 is followed by the ergosterol-dependent lateral movement of P2-bound Pex1p from ECR domains to ergosterol- and ceramide-poor domains. After their relocation to ergosterol- and ceramide-poor portions of the membranes of both fusion partners, P1-associated PI(4,5)P₂-bp and P2-bound PI(4,5)P₂-bp and Pex1p are released to the cytosol. Such release of PI(4,5)P₂-bp and Pex1p begins with a cytosol-dependent step, which is followed by a step that needs ATP hydrolysis. It remains to be established how the described remodeling of the peroxisome fusion machineries in the membranes of both fusion partners changes the physical properties and topology of lipid bilayers in which these machineries operate, thereby triggering peroxisome docking. By the end of the peroxisome docking process in *Y. lipolytica*, PI(4)P-bp and GTP-bp remain in ECR domains of the membrane bilayers of P1 and P2 vesicles. Several important observations have pointed to the possibility of Pex1p- and Pex6p-dependent membrane fusion acting in the multi-step process of peroxisome assembly in yeast species other than *Y. lipolytica*. First, similar to the effects of Pex1p and Pex6p deficiency reported for *Y. lipolytica* [22], deficiency in either of these two AAA ATPases results in the accumulation of small peroxisomal vesicles in *P. pastoris* [49,50] and *S. cerevisiae* [51], suggesting that vesicle fusion occurs also during the early steps of peroxisome assembly in these other
yeast species [19,20,52]. Second, similar to P1- and P2-bound Pex1p and Pex6p in *Y. lipolytica* [37,38,40], their counterparts in *P. pastoris* are associated with small peroxisomal vesicles that are distinct from mature peroxisomes [19]. Therefore, it has been suggested that the small pre-peroxisomal vesicles accumulated in Pex1p- or Pex6p-deficient *P. pastoris* cells could normally undergo successive rounds of fusion to generate larger vesicles [19,20]. It has been also proposed that these larger vesicles in *P. pastoris* cells could assemble the import machineries necessary for peroxisomal membrane and matrix proteins, import these proteins from the cytosol, and eventually mature into large, functional peroxisomes [19,20].

It seems that, while *Y. lipolytica* and, perhaps, *P. pastoris* Pex1p and Pex6p promote the fusion of early pre-peroxisomal vesicles, their *S. cerevisiae* counterparts function in the ATP-dependent recycling of the peroxisome-bound PTS1 receptor Pex5p back to the cytosol, thereby making it available for another round of protein import [53]. The recent assignment of this alternative function to *S. cerevisiae* Pex1p and Pex6p does not necessarily mean that these AAA ATPases play completely different roles in peroxisome assembly in different yeast species. Instead, it is feasible that Pex1p and Pex6p can actually operate in both processes, perhaps by driving peroxisome fusion at the very early, post-ER step of peroxisome assembly and by mediating the peroxisome-to-cytosol recycling of the PTS1 receptor during the late stages of the peroxisome maturation process. It is noteworthy that such a dual role for an AAA ATPase in yeast is not unprecedented. In fact, the AAA family ATPase Cdc48p/p97 has long been known to function in the homotypic fusion of ER membranes and in the assembly of the ER.
Golgi [54,55]. At the same time, Cdc48p/p97 has recently been shown to play a vital role in (1) the proteasome-dependent degradation of misfolded polyubiquitylated proteins that are exported from the ER through the ERAD pathway; (2) the proteasome-dependent activation and nuclear transport of some transcription factors; and (3) the rescaling and expansion of the nuclear envelope [56–58]. Importantly, while the essential role of Pex1p and Pex6p in peroxisomal membrane fusion has been established for the earliest intermediates of the peroxisome assembly and maturation pathway [37,38,40], the vital role of both these AAA ATPases in the ATP-dependent peroxisome-to-cytosol recycling of the PTS1 receptor has been demonstrated in the elegant series of experiments carried out with mature peroxisomes [53]. Altogether, these findings suggest that the currently available experimental data does not allow one to conclude that the AAA ATPases Pex1p and Pex6p play completely different roles in the multi-step processes of peroxisome assembly taking place in different yeast species.

4. Lipid transfer from the donor membranes of lipid bodies and ER expands the acceptor membranes of maturing peroxisomes

Peroxisomes in yeasts, plants and mammals lack enzymes required for the biosynthesis of their own membrane lipids [59–61]. Thus, any enlargement of peroxisomes that have been already detached from the ER template requires the transfer of lipids to their expanding membranes from the donor membranes of other organelles. What are these donor membranes? Lipid bodies, the ER-derived dynamic organelles consisting of a core of neutral lipids that are surrounded by a monolayer of glycerophospholipids and associated proteins [62], serve as the major source of membrane lipids for the expansion of peroxisomal membrane in germinated cotton oilseeds [60]. The postgerminative growth of these seeds results in dramatic enlargement of glyoxysomes, a distinct form of peroxisomes that is required for the conversion of storage oil into carbohydrates. Under these conditions, lipid bodies provide the expanding glyoxysome membranes with the bulk of neutral lipids, mostly triacylglycerols, and glycerophospholipids [60]. The transfer of triacylglycerols and glycerophospholipids from the donor membranes of lipid bodies to the acceptor membranes of glyoxysomes requires membrane proteins embedded into the glycerophospholipid monolayer surrounding lipid bodies [60]. The identity of these membrane proteins remains to be established. Importantly, although the ER membrane is the primary cellular location of glycerophospholipid-synthesizing enzymes and functions as the major site of glycerophospholipid biosynthesis [45,63], it does not serve as a donor membrane for the sorting of this class of lipids to the expanding glyoxysome membrane in germinated oilseeds [60].

In the yeast Yarrowia lipolytica, the expansion of the membranes of ER-derived immature peroxisomal vesicles is mandatory for their stepwise conversion to mature peroxisomes P6, which carry the complete set of membrane lipids [5,10,38,39]. The bulk of glycerophospholipids in this yeast is transferred from the donor membrane of a specialized sub-compartment of the ER to the closely apposed acceptor membranes of the immature peroxisomal vesicles P3 and P4 [38,66]. P3 and P4 are the early intermediates of the multistep pathway of peroxisome assembly operating in Y. lipolytica [38,39]. It seems that the transfer of glycerophospholipids from the donor membrane of this specialized sub-compartment of the ER to the acceptor membranes of P3 and P4 depends on the peroxisome-bound peroxin Pex2p. In fact, the pex2Δ knock-out mutation in Y. lipolytica significantly decreases the glycerophospholipid levels of membranes from P3 and P4 and simultaneously increases the level of membrane glycerophospholipids in the P3- and P4-associated sub-compartment of the ER [66]. This ER sub-compartment can be distinguished from the free form of the ER by buoyant density, the level of membrane glycerophospholipids, and protein spectrum [66]. Importantly, the pex2Δ mutation, which substantially decreases the level of membrane glycerophospholipids in P4, impairs the conversion of P4 to P5 [66]. Taken together, these findings suggest that the Pex2p-dependent transfer of glycerophospholipids from the P3- and P4-associated ER sub-compartment to the acceptor membranes of P3 and P4 provides these membranes with the bulk quantities of this lipid species and is essential for the conversion of P4 to the more mature peroxisomal vesicle, P5. The mechanism responsible for such ER-to-peroxisomal membrane transfer of glycerophospholipids remains to be established. It is conceivable that this transfer occurs at narrow cytosolic gaps called membrane contact sites [67], at which the ER and peroxisomal membranes come into close apposition. Recently, several working models for the role of ER-associated lipid-transfer proteins in the establishment and functioning of such membrane contact sites in yeast and mammalian cells have been proposed [67–71]. These models should serve as a useful starting point for examining such events during the multistep process of peroxisome assembly in Y. lipolytica.

5. Do membrane lipids regulate peroxisome division?

Peroxisomes in yeasts and humans do not grow and divide at the same time [1,39,72–74]. In the yeast Y. lipolytica, the growth of the immature peroxisomal vesicles P1 to P5, which is accomplished by the stepwise import of distinct subsets of matrix proteins and the uptake of lipids, and their development into mature peroxisomes, P6, occur before these completely
assembled mature peroxisomes undergo division [39]. The division of mature peroxisomes in *Y. lipolytica* is regulated by an unusual mechanism that controls membrane scission in response to a signal transmitted from inside the peroxisome [39]. The import of matrix proteins into different immature intermediates along the peroxisome assembly pathway provides them with an increasing fraction of the matrix proteins present in mature peroxisomes. This increase in the total mass of matrix proteins above a critical level causes the redistribution of a peroxisomal protein, acyl-CoA oxidase (Aox), from the matrix to the membrane [39]. A significant redistribution of Aox occurs only in mature peroxisomes, which contain the greatest percentage of matrix proteins. Inside mature peroxisomes, the membrane-bound pool of Aox interacts with Pex16p. Pex16p is a membrane-associated peroxin that in *Y. lipolytica* negatively regulates the membrane scission event required for the division of immature peroxisomal vesicles, thereby preventing their excessive proliferation [39,75]. The interaction between membrane-attached Aox and Pex16p terminates the negative action of Pex16p on scission of the peroxisomal membrane, thereby allowing mature peroxisomes to divide [32].

Like any event of membrane scission [45,76–79], scission of the peroxisomal membrane must be preceded by the destabilization of the membrane bilayer leading to membrane bending (Fig. 2). Bending and scission of mitochondrial, chloroplast and Golgi membranes in yeasts, plants and mammals are energetically unfavorable processes that require several teams of proteins [79–91] and a distinct set of membrane lipids, including phosphoinositides [77,82,83,92,93], phosphatidic acid (PA) [45,78,81,86,94] and diacylglycerol (DAG) [45,78,81,93]. Cone-shaped PA induces negative monolayer curvature in the cytosolic leaflet of a membrane bilayer in the constricted neck (Fig. 2; [45,78,82]). DAG, which has even more conical shape [78,82,95] and is capable of very rapid transbilayer...

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**Fig. 2.** The molecular shape of membrane lipids in various model organisms plays an important role in generating membrane curvature. The term “negative curvature” is used for defining the regions of a membrane bilayer that curve away from the cytosol, whereas the term “positive curvature” indicates membrane areas that curve in the direction of the cytosol. LPA has a shape of inverted cone as the cross-section area of its hydrophilic headgroup considerably exceeds that of its single hydrophobic tail. This inverted-cone-shaped lipid favors positive monolayer curvature. The molecular shape of LPA makes it ideally suited for fitting in the inner (lumenal) leaflet of a membrane bilayer in the constricted neck of a dividing organelle. In the cone-shaped lipids PA and DAG, the cross-section area of hydrophilic headgroup is smaller than that of their two hydrophobic tails. Therefore, PA and especially DAG induce negative monolayer curvature in the outer (cytosolic) leaflet of a membrane bilayer in the constricted neck of a dividing organelle. The molecular shape of lipids is not the only determinant of membrane curvature. Several groups of curvature-generating and curvature-sensing proteins [79,91] team up with membrane lipids in modulating the local curvature of organellar membranes.
movement and lateral partitioning [45, 67, 95–97], is a particularly potent inducer of negative monolayer curvature and membrane bending in various model organisms (Fig. 2; [77, 78, 95–97]). A major challenge now is to define the mechanism by which the Pex16p- and Aox-dependent intraperoxisomal signaling cascade promotes the membrane scission event required for peroxisome division in *Y. lipolytica*. Future work will aim at understanding how individual lipid species and lipid domains initiate the highly dynamic changes in the topology of the membrane bilayer in dividing peroxisomes, thereby generating negative membrane curvature and promoting destabilization, bending, scission and fission of the bilayer.

6. Future perspectives

Despite recent progress in our understanding of how the segregation of lipids into distinct domains in the peroxisomal membrane activates the multicomponent protein machinery serving peroxisome fusion in the yeast *Y. lipolytica*, we are still lacking answers to many important questions regarding the role of membrane lipids in regulating multiple steps of the peroxisome biogenesis process. Do peroxisomes in organisms and cell types other than *Y. lipolytica* and mouse dendritic cells undergo multistep assembly and maturation? Is the ER-derived peroxisomal endomembrane system unique to *Y. lipolytica* and mouse dendritic cells, which are which are capable of moving massive amounts of proteins through their secretory and endocytic endomembrane systems and are known for extensive peroxisome proliferation [21, 98–101], or do multiple peroxisomal subforms found in other organisms and cell types [14–20] constitute similar multi-compartmental endomembrane systems? How do lipids and lipid membrane domains of the peroxisomal membrane regulate the processes of peroxisome assembly, maintenance and propagation in organisms other than *Y. lipolytica*? How do ergosterol- and ceramide-rich lipid domains and the glycerophospholipid-enriched phase found in the ER membrane of yeast cells [36] control the observed lateral segregation of group I PMPs from secretory and ER resident membrane proteins within this membrane? What role individual lipid species, lipid domains and lipid–protein complexes in the ER membrane of yeasts could play in the selective packaging of group I PMPs into pre-peroxisomal carriers that are distinct from the ER-derived vehicles serving the intracellular trafficking of secretory and plasma membrane proteins? What peroxisomal membrane lipids and PMPs compose the molecular machinery that tethers and docks membranes of the ER-derived immature peroxisomal vesicles P1 and P2 in *Y. lipolytica*, thereby preparing them for fusion? Do, akin to membrane fusion reactions inside the secretory and endocytic systems of vesicular flow, tethering and docking of P1 and P2 require Q- and R-SNAREs or do these processes occur in a SNARE-independent fashion, similar to mitochondrial fusion? Do tethering and docking of P1 and P2 in *Y. lipolytica* result in the assembly of a “vertex” ring of distinct species of lipids and proteins, similar to the assembly of membrane lipids and proteins into the vertex ring structures on tethered yeast vacuoles? What is the molecular and functional organization of narrow cytosolic gaps called membrane contact sites [67–71], at which lipids in yeast, plant and mammalian cells are transferred from the donor membranes of lipid bodies and ER to the closely apposed acceptor membranes of peroxisomes that have been already detached from their ER-template? How do individual lipid species and lipid domains initiate the highly dynamic changes in the topology of the membrane bilayer in dividing peroxisomes of *Y. lipolytica*, thereby generating negative membrane curvature and promoting destabilization, bending, scission and fission of the bilayer? What is the mechanism by which these specific changes in membrane curvature promote the assembly of the protein machinery that executes the final steps of peroxisome division? Addressing these questions at the molecular level will ultimately provide greater understanding of the mechanisms regulating the highly dynamic processes of peroxisome assembly and maintenance.

Acknowledgments

This work was supported by grants from the Canadian Institutes of Health Research and the Natural Sciences and Engineering Council to V.I. Titorenko and by the Canada Graduate Scholarships Doctoral Award from the Canadian Institutes of Health Research to T. Boukh-Viner.

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