Peroxisome biogenesis: the peroxisomal endomembrane system and the role of the ER

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Peroxisomes have long been viewed as semiautonomous, static, and homogenous organelles that exist outside the secretory and endocytic pathways of vesicular flow. However, growing evidence supports the view that peroxisomes actually constitute a dynamic endomembrane system that originates from the endoplasmic reticulum. This review highlights the various strategies used by evolutionarily diverse organisms for coordinating the flow of membrane-enclosed carriers through the peroxisomal endomembrane system and critically evaluates the dynamics and molecular mechanisms of this multistep process.

Introduction

An important conceptual advance in our understanding of the basic principles of cellular organization is that the peroxisome, an organelle known for its essential role in lipid metabolism, is derived from the ER (Titorenko et al., 1997; Titorenko and Rachubinski, 1998; Mullen et al., 1999; Hoepfner et al., 2005; Kragt et al., 2005; Tam et al., 2005; Haan et al., 2006; Kim et al., 2006). A growing body of evidence also supports the view that peroxisomes, similar to the secretory endomembrane system of vesicular flow, constitute a multicompartmental endomembrane system in which individual compartments undergo a stepwise, time-ordered conversion into mature, metabolically active peroxisomes (Titorenko et al., 2000; Titorenko and Rachubinski, 2000; Geuze et al., 2003; Guo et al., 2003). All of these findings contradict the common textbook rendition of the peroxisome as a semiautonomous, static, and homogenous subcellular compartment whose assembly, as an organelle outside the secretory and endocytic pathways of vesicular flow, does not involve intercompartmental vesicular trafficking (Lazarow, 2003). Now, as this basic paradigm of cellular organization is about to be revised in cell biology textbooks (Kunau, 2005; Schekman, 2005),

there is an urgent need to recapitulate numerous observations on the dynamic nature of the ER-dependent process of peroxisome assembly. The scope of this paper is to summarize the growing evidence in support of a role for the ER as the template for the formation and maintenance of peroxisomes. We also discuss our current knowledge of the multistep, bidirectional flow of membrane-enclosed protein carriers through the ER-derived peroxisomal endomembrane system. In addition, we outline the most important unanswered questions and directions for future research in this vibrant and rapidly evolving field.

Targeting of peroxisomal membrane proteins (PMPs) to the ER and their sorting within the ER

The origin of peroxisomes has long been matter of debate, and partially underscoring this controversy has been the mode by which peroxisome-destined proteins are synthesized and targeted within the cell. For instance, a major tenant of the previous "ER-vesiculation" model for peroxisome biogenesis was that all of the soluble and membrane bound protein constituents of the peroxisome were synthesized cotranslationally on the ER (Beevers, 1979). These nascent proteins were proposed to then be sequestered into an expanding vesicle that would eventually bud from the ER to produce a mature, functional peroxisome (Beevers, 1979). However, subsequent observations suggested that peroxisomal proteins were not synthesized on the ER but on free polyribosomes in the cytosol. These and other data led to the "growth and division" model for peroxisome biogenesis wherein peroxisomes, like mitochondria and chloroplasts, were considered to increase in size by the posttranslational import of their protein constituents and proliferate only through the division of preexisting peroxisomes (Lazarow and Fujiki, 1985; Purdue and Lazarow, 2001; Lazarow, 2003). Notably, the ER in the "growth and division" model was deemed only to be a source of membrane lipids for the enlargement of preexisting peroxisomes.

Although for most of the past two decades the "growth and division" model has generally been considered the paradigm for peroxisome biogenesis, the recent monitoring of the sorting of various PMPs in evolutionarily diverse organisms has revealed that for at least a subset of these PMPs, referred to as group I PMPs (Titorenko and Rachubinski, 2001), the initial sorting site is the ER rather than the peroxisome membrane.

Sorting of group I PMPs to and within the ER also appears to be mediated by several different mechanisms (Fig. 1). For instance, in mammalian cells, the group I PMP Pex16p is inserted cotranslationally into ER membranes and seems to be localized throughout the entire ER before its sorting to peroxisomes (Kim et al., 2006). In the yeast *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, and *Hansenula polymorpha*, group I PMPs Pex2p, -3p, and -16p are also initially targeted to the "general" ER (Titorenko and Rachubinski, 1998; Hoepfner et al., 2005; Haan et al., 2006). However, unlike mammalian Pex16p, the ER targeting and insertion of these essential components of peroxisome assembly in *S. cerevisiae* does not require the Sec61p-dependent machinery for co- and posttranslational import of secretory proteins (South et al., 2001). Furthermore, unlike mammalian Pex16p that remains in the general ER before its sorting to peroxisomes (Kim et al., 2006), at least one of the group I PMPs in *S. cerevisiae*, namely, Pex3p, is directed from the general ER to a distinct subdomain of the ER (Hoepfner et al., 2005). This ER subdomain is referred to as the preperoxisomal template (Titorenko and Rachubinski, 2001) and is considered to be the site where preperoxisomal carriers are formed. That is, after being segregated into the preperoxisomal template, Pex3p serves as a docking factor for Pex19p, a predominantly cytosolic protein (Hoepfner et al., 2005). The Pex3p-dependent recruitment of Pex19p from the cytosol to the outer face of the preperoxisomal template in *S. cerevisiae* is mandatory for the budding of small preperoxisomal vesicles (Hoepfner et al., 2005). These ER-derived carriers of Pex2p, -3p, -16p, and -19p lack secretory cargo proteins (Titorenko et al., 1997).

Although the mechanism responsible for segregating group I PMPs from secretory and ER resident membrane proteins in yeast remains to be established, it is noteworthy that the membrane of the ER-derived preperoxisomal vesicles in *Y. lipolytica* has unusual ergosterol- and ceramide-rich lipid domains (Boukh-Viner et al., 2005). These lipid domains are similar to detergent-resistant lipid domains in the membrane of *S. cerevisiae* ER, where glycosylphosphatidylinositol-anchored secretory proteins cluster and thereby segregate from other secretory proteins (Mayor and Riezman, 2004). It is possible, therefore, that discrete lipid domains, perhaps ergosterol- and ceramide-rich lipid domains, in the membrane of yeast ER serve also as a sorting platform for segregating group I PMPs from secretory and ER resident membrane proteins. The resulting partitioning of group I PMPs into these membrane domains could also serve to generate an ER template for the formation of preperoxisomal vesicles.

In contrast to yeast Pex2p, -3p, and -16p and mammalian Pex16p, other group I PMPs, such as ascorbate peroxidase (APX) in plant cells (Mullen et al., 1999) and Pex13p in mouse dendritic cells (Geuze et al., 2003), can only be detected in a distinct portion of the ER, suggesting that they are targeted from the cytosol directly to a preexisting subdomain of the ER membrane. The terms peroxisomal ER (pER) and lamellar ER extension were coined for this ER found in plants and mice, respectively (Mullen et al., 2001; Tabak et al., 2003). At least one notable difference between these two ER subdomains is that pER is considered to be a portion of rough ER membrane (Lisenbee et al., 2003), whereas the lamellar ER extension is
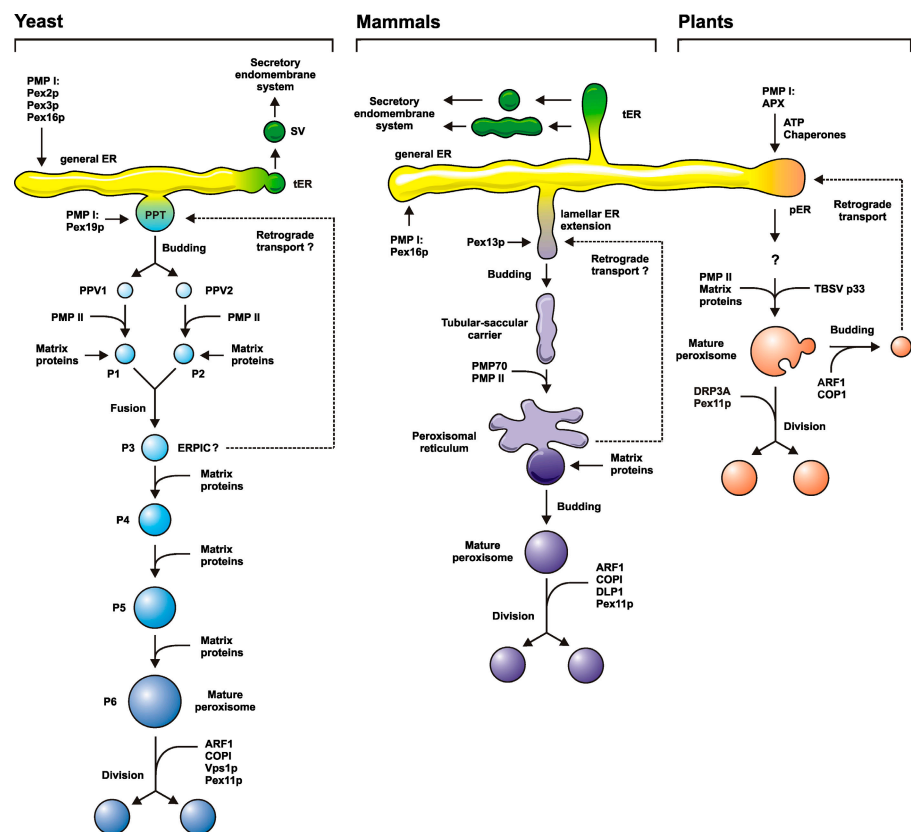
Figure 1. **Generalized models for the flow of membrane-enclosed carriers through the peroxisomal endomembrane system in yeast, mammals, and plants.** PPT, preperoxisomal template; PPV, preperoxisomal vesicle; SV, secretory vesicle; TBSV p33, TSBV 33-kD replicase protein.

a specialized domain in smooth ER membrane (Geuze et al., 2003). In plant cells, the cytosol-to-pER targeting of APX occurs posttranslationally and requires ATP as well as at least three components of the Hsp70 chaperone machinery (Mullen et al., 1999).

Collectively, the aforementioned findings suggest that by segregating a distinct set of membrane proteins and lipids into specialized ER subdomains, plant and mouse dendritic cells have evolved a platform for the targeting of group I PMPs from the cytosol to the ER membrane. The existence of such a platform in the ER membrane could increase the efficiency of the ER-dependent, multistep process of peroxisome assembly in these cells.

What structural features of group I PMPs are crucial for their sorting to the ER or to the peroxisomal membrane via either general ER or an ER subdomain remain to be determined. At present, it seems that the targeting of these PMPs from the cytosol to the ER membrane and their subsequent exit from the ER are mediated by two partially overlapping sets of sorting signals. One set of signals targets group I PMPs either co- or posttranslationally to the general ER or an ER subdomain, whereas the other set of signals act from within the ER lumen to sort these PMPs to the peroxisome (Baerends et al., 1996; Elgersma et al., 1997; Mullen and Trelease, 2000; Kim et al., 2006).

Exit of PMPs from the ER via preperoxisomal carriers

Although all group I PMPs exit the ER via distinct preperoxisomal carriers that do not enter the classical secretory pathway of vesicular flow (Titorenko and Rachubinski, 1998; Geuze et al., 2003), the morphology of these carriers in at least yeast and mammalian cells appears to differ (Fig. 1). In *S. cerevisiae*, *Y. lipolytica*, and *H. polymorpha*, the ER-derived preperoxisomal carriers are small vesicles (Titorenko and Rachubinski, 1998; Hoepfner et al., 2005; Haan et al., 2006). In contrast, the preperoxisomal carriers in mouse dendritic cells arise through direct en block protrusion of the specialized ER subdomain, the lamellar ER extension (Geuze et al., 2003). After reaching a considerable size, the lamellar extension detaches from the ER, giving rise to pleomorphic tubular-saccular carriers of group I PMPs. This detachment of preperoxisomal tubular-saccular carriers from the ER does not require coat protein complexes (COPs) I and II, which function in the formation of ER-derived carriers for secretory proteins (Geuze et al., 2003).

It is noteworthy that, akin to ER-derived preperoxisomal carriers in yeast cells, all known types of transport carriers for secretory proteins are small vesicles in these cells (Lee et al., 2004). On the contrary, just like the preperoxisomal carriers in mammalian cells, at least a subset of ER-to-Golgi carriers for many secretory proteins in these cells are pleomorphic tubular-saccular structures that are formed through direct en block protrusion of specialized domains in the ER membrane (Watson and Stephens, 2005). This fundamental difference in the morphology of ER-derived transport carriers is likely due to the difference in the spatial organization of transitional ER (tER), a specialized ER subdomain at which proteins are packaged into membrane-enclosed carriers. In the traditionally used model yeast organism, *S. cerevisiae*, the entire ER acts as tER, facilitating the budding of COPII-coated vesicles (Rossanese et al., 1999). In contrast, the tER of mammalian cells is organized into discrete ER export sites (Hammond and Glick, 2000). It is therefore possible that by segregating a distinct set of membrane proteins and lipids into a specialized ER subdomain for the cytosol-to-ER targeting of group I PMPs (see the previous section), higher eukaryotic organisms have not only separated these domains from the sites for the ER targeting of secretory proteins but also developed a platform for the sculpturing of these pER subdomains into pleomorphic tubular-saccular carriers of PMPs. A critical evaluation of this hypothesis would require testing the spatial organization of the ER subdomains for the cytosol-to-ER targeting of group I PMPs and examining the morphology of ER-derived carriers for these PMPs in the yeast *Pichia pastoris*. Unlike *S. cerevisiae* and similar to mammals, *P. pastoris* has discrete tER export sites that give rise to a "conventional" mammalian-type secretory apparatus (Rossanese et al., 1999).

Presently, no solid data exist for the nature of the preperoxisomal carriers in plant cells, although, similar to mammals, the tER in these cells is restricted to discrete sites in the ER membrane (Hanton et al., 2005), suggesting that the organization of the pER subdomain as well as the formation of preperoxisomal carriers in plants is similar to that in mammals.

Spatiotemporal organization of the peroxisomal endomembrane system

Recent findings have provided strong evidence that, analogous to some organelles of the secretory endomembrane system, peroxisomes constitute a dynamic organelle population consisting of many structurally distinct compartments that differ in their import competency for various proteins. Moreover, it appears that the individual compartments of this peroxisomal endomembrane system undergo a multistep conversion to mature peroxisomes in a time-ordered manner.

Two multistep pathways for peroxisome assembly and maturation have been described (Fig. 1). In *Y. lipolytica*, the posttranslational sorting of two partially overlapping sets of PMPs and a few matrix proteins converts two populations of ER-derived preperoxisomal vesicular carriers into the small (75–100 nm) peroxisomal vesicles P1 and P2 (Titorenko et al., 2000). These vesicles then serve as the earliest intermediates in a multistep pathway that involves, at each step, the uptake of lipids and the selective import of matrix proteins, eventually resulting in the formation of a mature peroxisome referred to as P6 (Guo et al., 2003). Overall, it seems that in *Y. lipolytica* and perhaps in other yeast, import machineries specific for different peroxisomal matrix proteins undergo a temporally ordered assembly in distinct vesicular intermediates along the peroxisome maturation pathway (Titorenko and Rachubinski, 2001). The plasticity of these import machineries is further underscored by the observation that the efficiency with which they recognize nonoverlapping targeting signals present on some of their protein substrates varies under different metabolic conditions. In fact, peroxisomal subforms present in yeast cells growing under

conditions that induce peroxisome proliferation differ from basal, nonproliferated subforms with respect to the targeting sequence motifs that are used to direct the same protein to these different subforms of peroxisomes (Wang et al., 2004).

A quite different scenario orchestrates a multistep process of peroxisome assembly and maturation in mouse dendritic cells. Herein, the extrusion of the lamellar ER extensions is culminated by the detachment of pleomorphic tubular-saccular carriers of Pex13p from the ER (Geuze et al., 2003). Only after their separation from the ER are these preperoxisomal carriers able to recruit to their membranes the ATP binding cassette transporter protein PMP70 and, perhaps, the membrane components of the import machinery for peroxisomal matrix proteins (Tabak et al., 2003). This latter step of the peroxisome maturation pathway also results in the formation of the so-called peroxisomal reticulum. Only the peroxisomal reticulum is capable of importing at least two peroxisomal matrix proteins, namely, thiolase and catalase, directly from the cytosol (Geuze et al., 2003). Notably, these two peroxisomal matrix proteins do not fill the entire peroxisomal reticulum. Instead, they are sorted exclusively into mature globular peroxisomes that, during the final step in the peroxisome maturation pathway in mouse cells, bud from the peroxisomal reticulum (Geuze et al., 2003; Tabak et al., 2003). It remains to be established whether other peroxisomal matrix proteins, similar to thiolase and catalase, are imported into the domain of the peroxisomal reticulum that gives rise to mature globular peroxisomes or whether, alternatively, these other matrix proteins in mouse cells are sorted to globular (mature) peroxisomes only after their budding from the peroxisomal reticulum.

In both models for the multistep assembly and maturation of peroxisomes, the targeting of PMPs 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. Because this strategy for peroxisome biogenesis has been conserved in the course of evolution, it seemingly provides an important advantage for the efficient, stepwise assembly of mature, metabolically active peroxisomes. It remains to be established whether, similar to a stepwise assembly of import machineries specific for different peroxisomal matrix proteins in yeast cells (Titorenko and Rachubinski, 2001), the import machineries for such proteins in mammalian cells can undergo a temporally ordered assembly in distinct intermediates along the peroxisome maturation pathway.

It is also unclear at the moment whether the peroxisome maturation pathway acting in mammalian cells, akin to the pathway that functions in yeast cells (Titorenko and Rachubinski, 2000; Titorenko et al., 2000), includes fusion of any early pathway intermediates. It is tempting to speculate that such fusion of early pathway intermediates in yeast results in the formation of an ER–peroxisome intermediate compartment (ERPIC). Such a compartment could (1) provide a template for the formation of downstream intermediates in the peroxisome assembly and maturation pathway and (2) function in the sorting of PMPs from those escaped ER resident proteins that are retrieved by retrograde vesicular transport between the ERPIC and the ER. Both of these tentative functions of the ERPIC share similarity

with the functions that have been proposed for the ER–Golgi intermediate compartment, also known as vesicular tubular clusters, which may regulate a bidirectional traffic of membrane-enclosed carriers through the classical secretory pathway (Lee et al., 2004). Importantly, the resident proteins of the post-ER compartments in both the peroxisomal endomembrane system and the classical secretory system return to the ER in response to the treatment of yeast cells with brefeldin A, an inhibitor of COPI formation (Salomons et al., 1997). Thus, similar to its role in the secretory endomembrane system, yeast COPI can function in the retrieval of those ER resident proteins that had entered the peroxisomal endomembrane system by mistake. This is in contrast to COPI in cultured human fibroblasts, in which peroxisome-to-ER retrograde protein transport, if any, does not depend on COPI (South et al., 2000; Voorn-Brouwer et al., 2001). These findings further support the notion that yeast and higher eukaryotic organisms may use different strategies for the ER-dependent formation and maintenance of their peroxisomal endomembrane systems.

Peroxisome-to-ER retrograde protein transport

Although it is not yet known whether, in plants, a multistep pathway for peroxisome assembly and maturation exits that is either similar or distinct from that in yeast and/or mammals, recent findings suggest that peroxisomes in plant cells can form large pleomorphic structures reminiscent of the mammalian peroxisomal reticulum (Mullen et al., 2006) and are engaged in ER-destined retrograde vesicular flow (Fig. 1). Evidence for this latter conclusion comes from observations that when the tomato bushy stunt virus (TBSV) replication protein p33 is expressed on its own in plant cells, it is sorted initially from the cytosol to peroxisomes and then, via peroxisome-derived vesicles and together with resident PMPs, to the pER (McCartney et al., 2005). Remarkably, several aspects of the peroxisome-to-pER sorting of p33- and resident PMP–laden vesicles are similar to the Golgi-to-ER retrograde vesicular transport. For instance, both these processes depend on the ADP-ribosylation factor (ARF) 1, which promotes the formation of COPI-coated vesicles (Lee et al., 2004; McCartney et al., 2005). In addition, the targeting signal of p33 that mediates the sorting of peroxisomal-derived vesicles to the pER resembles an arginine-based motif responsible for the COPI-dependent, vesicle-mediated retrieval of escaped ER membrane proteins from the Golgi (McCartney et al., 2005). Based on these and other observations, it has been suggested that the p33-promoted peroxisome-to-pER retrograde transport of vesicles delivers to the pER "early peroxins" (membrane bound peroxins involved in the early stages of peroxisomal membrane assembly) that stimulate the formation of membrane-enclosed carriers of PMPs as an essential phase of the TBSV life cycle (McCartney et al., 2005; Mullen et al., 2006). It is not clear at the moment whether the proposed reverse protein sorting pathway between peroxisomes and ER can only be induced in TBSV-infected plant cells or if it can also function in uninfected plants, or in other organisms, as a mechanism for the retrieval of escaped ER resident proteins.

Coordination of compartment assembly and division in the peroxisomal endomembrane system

In addition to their proposed role in the peroxisome-to-ER retrograde protein transport in virus-infected plant cells, both ARF1 and COPI can induce the proliferation of the peroxisomal endomembrane system in other evolutionarily diverse organisms by promoting the membrane scission event required for peroxisome division (Fig. 1). In fact, yeast mutants impaired in ARF1 and COPI, as well as mammalian cells deficient in COPI assembly, accumulate a reduced number of elongated tubular peroxisomes, consistent with impairment in peroxisome vesiculation (Passreiter et al., 1998; Lay et al., 2005). Incubation of highly purified rat liver peroxisomes with cytosol results in specific binding of both ARF1 and COPI to the peroxisomal membrane, further supporting the notion that their recruitment from the cytosol in living cells is an initial event in the proliferation of the peroxisomal endomembrane system (Anton et al., 2000). Moreover, similar to ARF1, the subtype 3 of yeast ARF also controls peroxisome division in vivo, although, in contrast to ARF1, in a negative fashion (Lay et al., 2005). Collectively, these findings suggest that the peroxisomal endomembrane system and the classical secretory system of vesicular flow are served by a similar set of core protein components required for their communication with the ER and for their proliferation. The proliferation of the individual compartments of the peroxisomal endomembrane system is also driven by a peroxisome-specific protein machinery, which includes a distinct set of the PMPs and the dynamin-related proteins DLP1 (dynamin-like protein 1), DRP3A (dynamin-related protein 3A), and Vps1p (vacuolar protein sorting protein 1), recruited from the cytosol to the peroxisomal surface by their receptor Fis1p (Thoms and Erdmann, 2005; Yan et al., 2005). A challenge for the future will be to define how the interplay of all these protein components governs such proliferation under the different metabolic conditions in a given cell type or tissue.

Importantly, peroxisome biogenesis appears to occur by way of a collaborative effort between two equally important pathways. The first pathway operates through the ER-dependent formation and maturation of the individual compartments of the peroxisomal endomembrane system, whereas the second pathway involves the precisely controlled division of these peroxisomal compartments. Growing evidence supports the view that cells have evolved at least two strategies for the coordination of compartment assembly and division in the peroxisomal endomembrane system. In the first strategy, the multistep growth and maturation of the ER-derived preperoxisomal carriers occurs before the completely assembled, mature peroxisomes undergo division (Guo et al., 2003). In the second strategy, a significant increase in the number of preperoxisomal carriers, either by their en masse formation from the ER (Kim et al., 2006) or by the proliferation of a few preexisting carriers (Veenhuis and Goodman, 1990; Guo et al., 2003), precedes the growth of these early peroxisomal precursors by membrane and matrix protein import and their conversion to mature, functional organelles containing a complete complement of peroxisomal proteins. Determining the relative contribution of these different

mechanisms in the formation of peroxisomes in any given organism should now be more feasible through the use of live-cell, photo/pulse-chase labeling methods similar to that reported recently for a study of peroxisome biogenesis in mammalian cells (Kim et al., 2006).

Regardless of the strategies that evolutionarily distant organisms use for coordinating the assembly and division of individual compartments of the peroxisomal endomembrane system, the tubulation, constriction, and scission of these compartments is regulated, depending on the cellular and/or environmental conditions of a particular cell type, either by signals emanating from within these compartments (Guo et al., 2003) or by extraperoxisomal signals that are generated inside the cell in response to certain extracellular stimuli (Yan et al., 2005). These intracellular signals include a distinct group of transcriptional factors that induce the transcription of genes encoding several proteins of the Pex11p family (Thoms and Erdmann, 2005). The peroxisome membrane bound Pex11p-type proteins then directly promote the proliferation of peroxisomal endomembrane compartments or activate peroxisome division indirectly by recruiting the dynamin-related proteins from the cytosol (Yan et al., 2005). Furthermore, the division of the individual compartments of the peroxisomal endomembrane system must be preceded by the expansion of their membranes because of the acquisition of lipids. The ER, a principal site for the biosynthesis of phospholipids, is the most likely source of lipids for the growth of the peroxisomal membrane (Purdue and Lazarow, 2001), although oil bodies have been implicated also as a source of peroxisomal lipids in some organisms, e.g., germinated oilseeds (Chapman and Trelease, 1991) and *Y. lipolytica* (Bascom et al., 2003). It seems that in *Y. lipolytica* the bulk of phospholipids is transferred from the donor membrane of a specialized subcompartment of the ER to the closely apposed acceptor membranes of the two early intermediates, P3 and P4, in the peroxisome assembly pathway (Titorenko et al., 1996). Although the mechanism responsible for such ER-to-peroxisomal membrane transfer of phospholipids via membrane contact sites remains to be established, several working models for the role of ER-associated lipid-transfer proteins in the establishment and functioning of such sites have recently been proposed (Levine, 2004). These models should serve as a useful starting point for examining such events during peroxisome biogenesis.

Conclusions and perspectives

Growing evidence supports the view that peroxisomes constitute a dynamic endomembrane system that originates from the ER. A major challenge now is to identify the molecular players that coordinate the flow of membrane-enclosed carriers through the peroxisomal endomembrane system. Future work will aim at understanding the spatiotemporal dynamics and molecular mechanisms underlying this multistep process in evolutionarily diverse organisms. It is conceivable that the analysis of a variety of model organisms, including tissue-cultured human cell lines and various yeast and plant species, will reveal as-yet-unknown strategies and mechanisms governing the biogenesis of the peroxisomal endomembrane system and its relationship with the ER.

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