# Peroxisome biogenesis and maintenance in yeast

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# Introduction: Peroxisome proliferation and dynamics

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## Abstract

Peroxisomes are organelles occurring in almost all eukaryotic organisms. Their structure is relatively simple - peroxisomes are composed of a single membrane enclosing a matrix containing a variety of enzymes. This rich enzymatic content makes peroxisomes important players in various cellular pathways. The hallmark of peroxisomes is their ability to dynamically adapt to changing conditions by adjusting their size, number and enzyme components. In yeast peroxisomes are formed either by fission of pre-existing ones or by a *de novo* pathway involving the endoplasmic reticulum. An organelle inheritance system exists that allows delivery of peroxisomes to nascent buds. In this chapter the current knowledge of the molecular mechanisms involved in peroxisome formation and inheritance in yeast is summarized.

## Introduction

Eukaryotic cells contain morphologically and functionally diverse compartments called organelles. These membrane-bound structures provide specific micro-environments for distinct chemical reactions to take place, allowing co-existence of different processes within a cell. Peroxisomes form a functionally important class of organelles and are present in almost all eukaryotic cells. They consist of a single lipid bilayer enclosing the proteinaceous matrix filled with various enzymes. As peroxisomes do not contain DNA, all their components are encoded by nuclear DNA and synthesized in the cytosol. Peroxisomes are highly dynamic in their nature - they are able to adjust their number, size and enzyme content in response to internal and environmental stimuli.



A general function of peroxisomes is providing a compartment for metabolic reactions that lead to the formation of reactive oxygen species (ROS). Peroxisomes contain oxidases producing hydrogen peroxide, which is subsequently decomposed by an enzyme called catalase, residing in the peroxisomal matrix as well. Yeast peroxisomes are mainly specialized to metabolize unusual carbon and nitrogen sources such as oleic acid, methanol, D-amino acids and purines [1]. Proliferation of peroxisomes is strongly induced upon a shift of glucose-grown yeast cells to media containing one of these components as sole carbon or nitrogen source. Other examples of specialized functions of peroxisomes include the synthesis of plasmalogens and bile acids in human [2]. In plants they are implicated in the glyoxylate cycle and photorespiration [3]. Interestingly, in mammals peroxisomes are also involved in some non-metabolic functions such as providing antiviral innate immunity [4].

Biogenesis of peroxisomes is dependent on *PEX* genes. Over 35 *PEX* genes have been functionally analyzed revealing that most of them are implicated in the import of matrix enzymes. Other *PEX* genes are required for the insertion of peroxisomal proteins in the membrane or in the regulation of size and number of these organelles. The importance of peroxisomes in humans is highlighted by occurrence of severe disorders caused by their dysfunction, which may be an effect of either a mutation in one of the *PEX* genes (Peroxisomal Biogenesis Disorders - PBDs) or caused by single peroxisome enzyme deficiencies. Yeast offer an ideal model system for studies of peroxisome biogenesis, as, in contrast to human, mutations in *PEX* genes are not lethal in these organisms.

The molecular mechanisms involved in the biogenesis of peroxisomes are currently under debate. One of the models explaining peroxisome formation states that they are autonomous organelles able to undergo self-replication (similarly to mitochondria and chloroplasts). However, emerging

studies support an alternative model proposing that peroxisomes belong to the endomembrane system and may form *de novo* from the endoplasmic reticulum (ER) membrane. In this chapter we present an overview of our current knowledge on peroxisome formation and inheritance in yeast.

## Peroxisome proliferation by growth and fission

According to the classical model of peroxisome proliferation, these organelles form by growth and fission of pre-existing ones [5]. Peroxisomes may also form *de novo* from the ER [6], however, it is still debated whether this process occurs at normal conditions in wild type (WT) cells or only in mutant cells that temporarily lack peroxisomes (e.g. due to an inheritance defect).

The model of peroxisome growth and fission proposes that peroxisomes increase their size by importing newly synthesized matrix proteins from the cytosol, which is accompanied by incorporation of membrane lipids and peroxisomal membrane proteins (PMPs). When peroxisomes reach a certain size, their division is initiated. This process requires an orchestrated action of a set of proteins and takes place in three subsequent steps: organelle elongation, constriction and scission (Figure 1, steps 2-5).

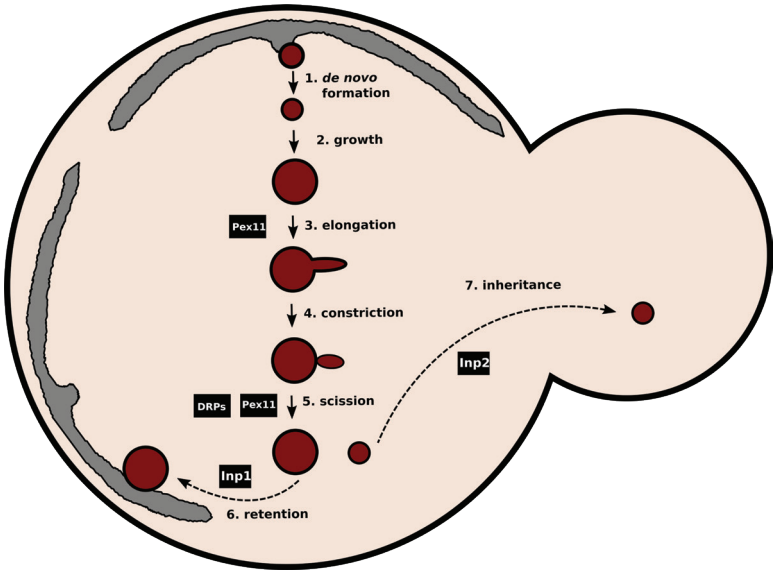


**Figure 1. A hypothetical model of peroxisome proliferation and inheritance in yeast.** In WT yeast cells peroxisomes proliferate mainly by growth (**step 2**) and fission (**steps 3 - 5**) of pre-existing organelles. Pex11 is considered a key component of the peroxisome fission machinery as it is implicated in different stages of this process. Peroxisomes may also form *de novo* from the ER, especially in cells temporarily devoid of these organelles (**step 1**). A newly formed peroxisome may be either retained in the mother cell (**step 6**) or transferred to the nascent bud (**step 7**) during cell division. Inp1 and Inp2, respectively, are involved in these events.

Pex11 is a key player in peroxisome fission as it is involved in the elongation of peroxisomes as well as in the final step of membrane scission [7,8]. Pex11 is the most abundant protein of the peroxisomal membrane [9]. Its N-terminal amphipathic α-helix (Pex11-Ampf) has been shown to have the ability to induce membrane curvature leading to membrane tubulation [10]. Recent studies in fungi have suggested that Pex11-Ampf oligomerization constitutes a prerequisite for membrane curvature [11]. This supports previous indications of a role of human Pex11β oligomerization in membrane remodeling [12]. Pex11 has been shown to be phosphorylated in different yeast species, however, the function of this post-translational modification is not conserved, because phosphorylation of Pex11 leads to stimulation of peroxisomal fission in *Saccharomyces cerevisiae* [13] and *Pichia pastoris* [14], but not in *Hansenula polymorpha* [15].



Next to the involvement in proliferation of peroxisomes, Pex11 was suggested to play a role in transport processes. Data obtained in *S. cerevisiae* indicates that Pex11 is important for transfer of medium chain fatty acids across the peroxisomal membrane [16]. Recent studies have revealed that Pex11 forms a non-selective pore that serves in exchanging metabolites across the peroxisomal membrane. Interestingly, phosphorylation of Pex11 affects its pore-forming activity and regulates the rates of β-oxidation [17]. Also, Pex11 levels are strongly linked to the rate of peroxisomal β-oxidation. In the absence of Pex11 this process is nearly fully blocked while overproduction of Pex11 leads to an increase of the β-oxidation rate [16].

Even though the yeast machinery responsible for the organelle constriction process is yet unknown, several proteins implicated in the final step of membrane scission were identified. These proteins include members of the dynamin related proteins (DRPs) family. DRPs are large GTPases that contain three conserved domains: a GTPase domain, a middle domain and a GTPase - effector domain [18]. The DRPs Vps1 and Dnm1 are important for peroxisome fission in *S. cerevisiae* [19], whereas in *H. polymorpha* only Dnm1 is a crucial protein involved in peroxisome fission [20]. Interestingly, the N-terminal domain of Pex11 can function as GTPase activation protein (GAP) for Dnm1 [8].

### Incorporation of membrane lipids

Yeast peroxisomes lack phospholipid biosynthesis enzymes, therefore, peroxisomal membrane lipids have to be delivered to these organelles from other sources. Most of the peroxisomal membrane lipids are synthesized at the ER. Studies using *S. cerevisiae* revealed that other organelles are involved as well, because phosphatidylethanolamine can be delivered to the peroxisomal membrane additionally from mitochondria and the Golgi apparatus [21]. A

mitochondrial origin of membrane lipids is also suggested by the observation that peroxisomal membranes isolated from *P. pastoris* contain cardiolipin, which is synthesized exclusively in mitochondria [22].

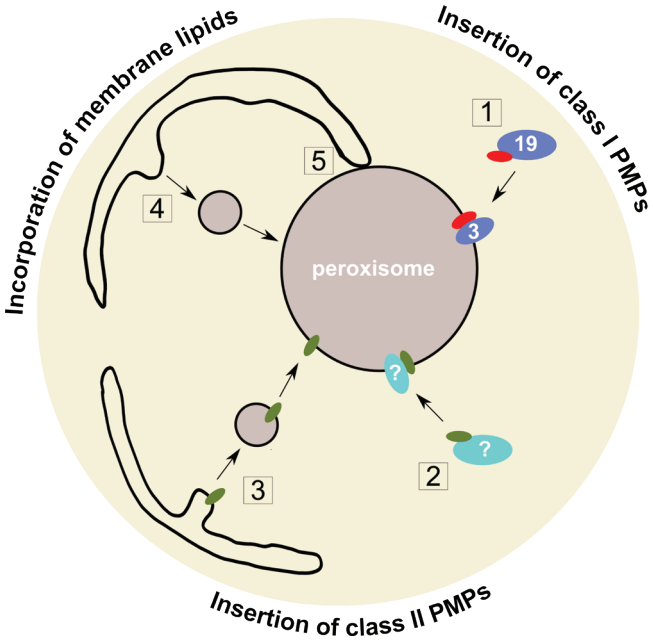


**Figure 2. A hypothetical model of peroxisome membrane formation.** Class I PMPs are inserted into the peroxisomal membrane via the Pex3/Pex19 complex (**1**). The remaining PMPs comprising class II may be inserted by other protein/protein complex (**2**) that has to be yet identified. The ER may serve as a lipid source for the growing peroxisomal membrane by their delivery either in form of ER-derived vesicles (**4**) or via contact sites (**5**). PMPs of class II may also traffic through the ER and exit that compartment in form of vesicles, which then could fuse with the pre-existing peroxisome, delivering both proteins and lipids to the growing peroxisomal membrane (**3**).

Two mechanisms of lipid transport to peroxisomes have been proposed. One of them implies that lipids reach the peroxisomal membrane in vesicles that pinch off from other cellular membranes (Figure 2, step 4). Such vesicular transport was reported for *Yarrowia lipolytica* [23]. Another possibility is that lipids are delivered to peroxisomal membranes via non-vesicular transport. It was first shown by the Prinz' group that lipids can be directly transferred from the ER to peroxisomes [24]. Non-vesicular lipid transport possibly takes place at membrane contact sites (MSCs) (Figure 2, step 5). MCSs are regions where two membranes come into close apposition, enabling exchange of small molecules between the two compartments [25]. Peroxisomes form contact sites with different membranes. Possibly, these regions may serve as sites facilitating transport of lipids.

So far, two contact sites between the ER and peroxisomes (EPCONS - ER-Peroxisome CONtact Site) have been identified in yeast. One of them has been described as a tether involving Pex3 protein that is present both at the ER and at peroxisomal membrane. Pex3 localized to these two compartments is bridged by a peripheral peroxisomal protein Inp1 [26]. This contact site has a well-established role in peroxisome retention in the mother cell during cell division. However, it cannot be excluded that it may also contribute to lipid transfer allowing expansion of the peroxisomal membrane.

Pex30 is a component of the second known yeast EPCONS and forms a complex with three reticulon-like proteins localized at the ER: Rtn1, Rtn2 and Yop1. This EPCONS has been proposed to serve as site of *de novo* peroxisome biogenesis enabled by changes in the ER architecture, triggered by the reticulon-like proteins [27,28]. Apart from this function, this contact site is a good candidate to serve as site providing pre-existing peroxisomes with lipids. This is evident for mammals, where interactions between peroxisomes and the ER are required for the synthesis of several lipids. The peroxisomal membrane protein ACBD5 (acyl-coenzyme A–binding domain protein 5) has been reported to act as a tether that interacts with the ER protein VAPB (vesicle-associated membrane protein-associated protein B) [29,30]. ACBD5 belongs to the ACBD family, characterized by the presence of an acyl-CoA binding domain. The ACBD5/VAPB association leads to the formation of MCSs between peroxisomes and the ER which facilitate transport of lipids, required for the growth of the peroxisomal membrane and plasmalogen synthesis. Recently, an additional ER-peroxisome tethering complex has been described, namely ACBD4/VAPB [31]. ACBD4 is a member of the same family as ACBD5 and displays sequence similarity restricted to the acyl-CoA binding domain. The presence of distinct types of tethers may reflect different roles fulfilled by their components. Considering a probable role of contact sites in mediating molecule transfer, differences between protein content of those contact sites may serve to extend the range of substrate specificity.

In *S. cerevisiae* two contact sites between the peroxisomes and mitochondria have been described. First, peroxisomes were observed to localize in proximity to the ERMES complex (ER-Mitochondria Encounter Structure) and sites of mitochondrial acetyl-CoA synthesis [32]. In line with this observation, a genome-wide high-content microscopy study resulted in the identification of physical contacts between peroxisomes and mitochondria, which involve the interaction between Pex11 and Mdm34, a mitochondrial component of ERMES [33]. Recent systematic studies aiming at identifying novel organelle contacts have also revealed the occurrence of a peroxisome-mitochondria contact site (PerMit), which is mediated by at least two tethers. One of these tethers

contains a mitochondrial protein Fzo1, and the other, a peroxisomal membrane protein Pex34 [34]. Their interacting partners at the opposing membranes are not yet known. These contacts serve as sites for metabolite exchange between the two compartments during β-oxidation. Although there is no evidence pointing to a role of PerMit in membrane lipid transfer, such function cannot be excluded and may be important to provide the peroxisomal membrane with cardiolipin.

### Formation of peroxisomes *de novo*

Even though fission seems to be a prevailing mode of peroxisome formation, at least in WT yeast cells, there is data suggesting that peroxisomes may be also formed *de novo* from the ER (Figure 1, step 1). The first observation linking peroxisome biogenesis with the ER was made in *Y. lipolytica*. In this yeast Pex2 and Pex16 are N-glycosylated suggesting their trafficking to the peroxisomal membrane through the ER compartment [35]. Biogenesis of peroxisomes from the ER was supported further by *in vitro* budding assays demonstrating that vesicles containing PMPs can bud from the ER [36,37]. The formation of vesicles *in vitro* required ATP, cytosolic factors and Pex19 [36], but was independent of Pex3 [37]. Although not yet experimentally proven i*n vivo*, such ER derived vesicular structures could subsequently fuse with each other, with pre-existing peroxisomes or grow into mature organelles.

Most of the evidence for the ER involvement in peroxisome biogenesis comes from the studies of Pex3- or Pex19-deficient yeast strains, which had been long considered to be devoid of any peroxisomal membrane structures. It was shown that upon reintroduction of the missing genes the peroxisome population could be restored in these mutants. Because newly produced Pex3 in *S. cerevisiae* was spotted at the ER before reaching peroxisomes, the ER was indicated to be the most probable template for formation of peroxisomes *de novo* [6,38]. The ER involvement in peroxisome formation in *S. cerevisiae* was further supported by the identification of a Pex3 domain responsible for targeting of this PMP to the ER. There, Pex3 accumulates at specialized regions where the formation of peroxisomes commences [39]. Another study revealed that a large set of PMPs localizes to the ER in *S. cerevisiae* [40]. These authors propose that PMPs exit the ER in two types of membrane vesicles which develop into metabolically active peroxisomes upon fusion and subsequent import of matrix enzymes from the cytosol. However, these observations should be interpreted carefully, keeping in mind that the studied proteins were expressed under control of strong promoters. It is known that overproduction of proteins often leads to their mislocalization to different cellular compartments [41]. In line with this, PMPs have not been so far reported to reside at the ER in WT cells in normal conditions. It is under debate whether Pex3 and other PMPs

always traffic to peroxisomes through the ER. Results obtained for mammalian cells indicate that newly synthesized Pex3 targets pre-existing peroxisomes directly [42].

The *de novo* formation model has been also challenged by recent findings related to pre-peroxisomal vesicles (PPVs) - structures that are present in Pex3-deficient mutants of *H. polymorpha* [43]. These vesicles contain a subset of PMPs and mature into functional peroxisomes upon reintroduction of Pex3. The fact that PPVs are located in close proximity of the ER may have led to previous incorrect conclusions about the ER localization of some of the tested PMPs. Detailed electron microscopy analysis showed clearly that these structures are separate from the ER compartment. However, it is still a plausible option that PPVs originate from the ER. PPVs have also been observed in Pex3-deficient *S. cerevisiae* cells (this thesis, Chapter 2, [44]). Joshi and colleagues proposed that PPVs originate from domains of the ER, to which Pex30 localizes specifically [45]. Interestingly, these Pex30-enriched domains also represent sites of lipid droplet biogenesis [46]. Recently, the (ESCRT)-III (endosomal sorting complexes required for transport) machinery has been shown to perform the scission step necessary to release pre-peroxisomal structures from the ER [47].



It is possible that in other species *de novo* formation plays a more prominent role in peroxisome biogenesis, as was often reported for higher eukaryotes [48,49]. Recent results point out the possibility of the involvement of mitochondria in the process of peroxisome biogenesis in human cells. Sugiura and colleagues proposed that peroxisomes form as a result of fusion between two types of vesicles - one derived from the ER and the second originating from mitochondria [50].

### PMPs sorting and insertion

The mechanisms of peroxisomal proteins insertion into the membrane are unknown. It is still under debate whether PMPs are directly inserted into the membrane upon their synthesis in the cytosol or they traffic via the ER.

According to the classical model, Pex19 acts as a soluble receptor for a variety of proteins that belong to the class I of PMPs. The C-terminal α-helical domain of Pex19 binds class I PMPs at their membrane targeting signal (mPTS) [51]. This receptor-cargo complex is then recruited to the peroxisomal membrane by Pex3, which is a docking site for Pex19, interacting with its N-terminal region [52,53]. Next, class I PMPs are inserted into the membrane via a yet unresolved mechanism (Figure 2, step 1). Class II PMPs are not recognized by Pex19. Yeast peroxins that are found within the second class of PMPs include Pex3 and Pex22. Pex3 contains peroxisomal

targeting information within its N-terminal domain. This region of Pex3 does not interact with Pex19 [52] and shares similarity with the N-terminal part of Pex22. When the N-terminal domain of Pex3 was replaced by the one of Pex22, no functional implications were observed in terms of Pex3 targeting and its role in peroxisome formation [54]. These findings confirm that Pex3 and Pex22 share an mPTS of the same nature that enables Pex19-independent targeting to the peroxisomal membrane. The machinery involved in recognition and recruitment of class II PMPs to the peroxisomal membrane may require proteins which have to be yet identified (Figure 2, step 2). An alternative possibility for Pex19-independent PMPs sorting is their trafficking to the peroxisomal membrane via the ER, mediated by ER-derived vesicles. Class II PMPs could enter the ER and then pinch off from the ER membrane as vesicles that could subsequently fuse with the growing peroxisomal membrane (Figure 2, step 3).

ER-derived vesicles have been described as precursors of mature peroxisomes in a model proposed by van der Zand and colleagues. The model implies that all PMPs first sort to the ER and next exit this compartment in form of two types of vesicles. The vesicles contain the components of either the docking complex or the RING finger complex of the peroxisomal importomer. In this scenario, Pex3 and Pex19 were suggested to function in the release of the vesicles from the ER. Subsequently, the distinct vesicles fuse with each other - a process dependent on Pex1 and Pex6. This leads to the assembly of the complete and functional importomer followed by import of matrix proteins [55].

A possible origin of peroxisomes from the ER is suggested by the fact that the PMPs Pex2 and Pex16 of *Y. lipolytica* undergo N-glycosylation [35] - a posttranslational modification that is restricted to the ER lumen. Therefore, it was proposed that *Yl*Pex2 and *Yl*Pex16 most probably traffic via the ER on their way to peroxisomal membrane. The mechanism of PMPs insertion into the ER membrane still needs to be evaluated since there is conflicting data regarding dependence of this process on the key component of protein translocation machinery from the ER, namely Sec61 [40,56].

Even though evidence exists for the majority of the PMPs to be inserted into the peroxisomal membrane directly from the cytosol, we cannot ignore the fact that some PMPs may traffic via the ER. The details of peroxisomal membrane protein assembly mechanisms need to be further investigated. Figure 2 represents a hypothetical model illustrating possible sorting pathways for PMPs and lipids.

### Inheritance

Yeast have to replicate their organelles and partition them between mother and daughter cells during cell budding. This process ensures that the mother cell keeps a necessary copy of organelles while the nascent cell obtains its fair share as well. The process of peroxisome inheritance can be, therefore, divided into two different actions, which are organelle retention and transport, involving different factors, such as motor, anchor and adaptor proteins.

Retention of peroxisomes in yeast mother cells is facilitated by a peripheral peroxisomal protein - Inp1 (Figure 1, step 6). Inp1 serves as an anchor connecting peroxisomes to the cell periphery, facilitating their retention in the mother cell during cell division. This role is accomplished by tethering peroxisomes to the ER, that occurs via the Inp1- Pex3 interaction [26]. Overexpression of *INP1* resulted in the absence of peroxisomes in most of the newly formed buds. On the other hand, deletion of *INP1* led to the presence of mother cells devoid of peroxisomes [57]. This disturbed distribution of peroxisomes, in cells overproducing or lacking Inp1, points to the importance of Inp1 in the process of peroxisome segregation between mother and daughter cells during budding. Interestingly, studies in *H. polymorpha* revealed that deletion of *PEX11* resulted in a similar retention defect as the one observed in the *inp1* mutant cells. During growth on glucose, all the peroxisomes were transferred to the buds of *pex11* mutant cells, rendering mother cells devoid of these organelles. This suggests a function of Pex11 in the retention of peroxisomes in the mother cell during cell division, as Inp1 is still properly targeted to peroxisomes in the absence of Pex11 [58].

The retention event is balanced by transport of peroxisomes to the forming bud (Figure 1, step 7). This process takes place via peroxisome movement along actin filaments. It involves several proteins performing different functions. The motor protein implicated in transport of peroxisomes to yeast daughter cells is the class V myosin - Myo2 [59]. It facilitates actin-based motion of peroxisomes by binding actin through its N-terminal domain. The C-terminal part of Myo2 has the ability to bind to cargo organelle containing the specific adaptor protein. During peroxisome transport Myo2 is recruited to the peroxisomal membrane by the adaptor Inp2 - an integral protein of peroxisomal membrane [60]. It has been reported that *S. cerevisiae* cells lacking Inp2 are not able to segregate peroxisomes to the buds [61]. As peroxisomes do not move from the mother cells, the newly formed daughter cells are initially devoid of peroxisomes and have to form them *de novo*.

*S. cerevisiae* Pex19 is also implicated in the inheritance process via its role in the formation of Myo2 - Inp2 complexes. Pex19 displays binding affinity to the C-terminal cargo binding domain of Myo2. Moreover, a Myo2 mutant with reduced capability to bind Pex19, but not Inp2, displayed an impaired peroxisome inheritance pattern. This observation provides yet another evidence for the multifunctional character of Pex19, besides its crucial role in peroxisome formation.

Studies in filaments fungi unraveled a very unique system of peroxisome inheritance involving formation of a contact site between peroxisomes and early endosomes. It has been shown that in *Aspergillus nidulans* a small portion of the peroxisomal population is transported by "hitchhiking" on early endosomes. The contact between these two organelles is mediated by the endosomal linker protein - PxdA. It cannot be excluded that other organisms may use similar systems, at least for transport of a fraction of peroxisomes to the nascent cells.

The peroxisome population of a cell is heterogeneous in terms of the organelle's relative age [64,65]. Mammalian peroxisomes within one cell display different capacities to import newly synthesized matrix proteins, with Pex14 being more abundant in younger organelles [64]. Yeast cells also contain both young and old organelles. Interestingly, it has been recently shown that the inheritance pattern is strictly related to the age of peroxisomes. During yeast cell division, the older organelles are preferentially retained in the mother cells, supporting selective transport of younger peroxisomes to the buds [65].

Peroxisome inheritance has to be precisely regulated in order to reach synchronization with the cell cycle. There is evidence suggesting that such regulation may be achieved via reversible post-translational modifications, such as phosphorylation of the inheritance machinery components. As an example, Inp2 undergoes phosphorylation, reaching the highest levels of this modification at the beginning and at the end of cell cycle [60]. This suggests that phosphorylation of Inp2 serves as a signal for its degradation once the protein is no longer required.

### Outlook

The strongly debated topic of peroxisome biogenesis still raises many controversies. There is a lot of data available on the molecular mechanisms of import of matrix proteins from the cytosol into peroxisomes [66], however, the processes involved in the formation of peroxisomal membranes are still largely unclear.

According to the current model, fission is the main mode of peroxisome proliferation, at least in WT yeast cells [61]. Convincing support for this model exists. The machinery involved in peroxisome division has been extensively described [19,67-69]. Additionally, there are several yeast mutants defective in fission that display very obvious phenotypes (e.g. *pex11*, *dnm1*, *vps1*). Yet, the contribution of peroxisome fission to peroxisome multiplication is not fully clear. While fission could be a sole form of peroxisome formation, we cannot ignore the data supporting formation of peroxisomes *de novo* from the ER.

There has been a lot of controversy on the sorting pathways of PMPs. Some results point to the ER as an intermediate compartment during PMPs trafficking to the peroxisomal membrane [6]. On the other hand, the Pex3/Pex19 machinery has a well-established function in targeting PMPs to their destination membrane [51-53]. It is tempting to speculate that these two processes may coexist in WT cells. They may be also dependent on the studied organism and genetic background. Most of the data that suggest trafficking of PMPs through the ER come from studies with peroxisome-deficient mutants - *pex3* and *pex19* [38,40,70]. In these cells PMPs may be targeted to alternative compartments as a result of peroxisome absence, which makes the native destination membrane unavailable for insertion. Even if peroxisomal membrane vesicles are present in mutant strains, not all PMPs may sort to these structures because of their relatively small dimension, limiting the surface available for PMPs incorporation. Peroxisomal vesicles membranes may get saturated quickly, causing the remaining PMPs, produced in excess, to be mistargeted to other compartments. In contrast, the peroxisomal membrane in WT cells expands by incorporation of lipids, constantly providing a new template for direct insertion of PMPs from the cytosol.

Further studies need to be conducted in order to decide whether peroxisomes belong to the endomembrane system or represent semi-autonomous organelles that only self-replicate. It would be of great value to isolate mutants that are completely devoid of any peroxisomal membranes. Such mutants would serve as a perfect tool for investigation of the mechanisms of peroxisomes/membrane vesicles biogenesis.

## References


1. van der Klei, I.J., Veenhuis, M. Yeast peroxisomes: function and biogenesis of a versatile cell organelle. *Trends Microbiol*. 1997; 5: 502–9. doi: 10.1016/S0966-842X(97)01156-6.
2. Wanders, R.J.A, Waterham, H.R. Biochemistry of mammalian peroxisomes revisited. *Annu Rev Biochem*. 2006; 75: 295–332. doi: 10.1146/annurev.biochem.74.082803.133329.
3. Reumann, S., Weber, A.P.M. Plant peroxisomes respire in the light: some gaps of the photorespiratory C2 cycle have become filled--others remain. *Biochim Biophys Acta*. 2006; 1763: 1496–510. doi: 10.1016/j.bbamcr.2006.09.008.
4. Odendall, C., Dixit, E., Stavru, F., Bierne, H., Franz, K.M., Durbin, A.F., et al. Diverse intracellular pathogens activate type III interferon expression from peroxisomes. *Nature Immunology*. 2014; 15: 717–26. doi: 10.1038/ni.2915.
5. Lazarow, P.B., Fujiki, Y. Biogenesis of peroxisomes. *Annu Rev Cell Biol.* 1985; 1: 489–530. doi: 10.1146/annurev.cb.01.110185.002421.
6. Hoepfner, D., Schildknegt, D., Braakman, I., Philippsen, P., Tabak, H.F. Contribution of the endoplasmic reticulum to peroxisome formation. *Cell*. 2005; 122: 85–95. doi: 10.1016/j.cell.2005.04.025.
7. Koch, J., Pranjic, K., Huber, A., Ellinger, A., Hartig, A., Kragler, F., et al. PEX11 family members are membrane elongation factors that coordinate peroxisome proliferation and maintenance. *J Cell Sci.* 2010; 123: 3389–400. doi: 10.1242/jcs.064907.
8. Williams, C., Opalinski, L., Landgraf, C., Costello, J., Schrader, M., Krikken, A.M., et al. The membrane remodeling protein Pex11p activates the GTPase Dnm1p during peroxisomal fission. *Proc Natl Acad Sci U S A*. 2015; 112: 6377–82. doi: 10.1073/pnas.1418736112.
9. Erdmann, R., Blobel, G. Identification of Pex13p a peroxisomal membrane receptor for the PTS1 recognition factor. *J Cell Biol*. 1996; 135: 111–21. doi: 10.1083/jcb.135.1.111.
10. Opaliński, Ł., Kiel, J.A.K.W., Williams, C., Veenhuis, M., van der Klei, I.J. Membrane curvature during peroxisome fission requires Pex11. *EMBO J.* 2011; 30: 5–16. doi: 10.1038/emboj.2010.299.
11. Su, J., Thomas, A.S., Grabietz, T., Landgraf, C., Volkmer, R., Marrink, S.J., et al. The N-terminal amphipathic helix of Pex11p self-interacts to induce membrane remodelling during peroxisome fission. *Biochim Biophys Acta Biomembr*. 2018; 1860: 1292–300. doi: 10.1016/j.bbamem.2018.02.029.
12. Bonekamp, N.A., Grille, S., Cardoso, M.J., Almeida, M., Aroso, M., Gome,s S., et al. Self-interaction of human Pex11pβ during peroxisomal growth and division. *PloS One*. 2013; 8: e53424. doi: 10.1371/journal.pone.0053424.
13. Joshi, S., Agrawal, G., Subramani, S. Phosphorylation-dependent Pex11p and Fis1p interaction regulates peroxisome division. *Mol Biol Cell*. 2012; 23: 1307–15. doi: 10.1091/mbc.E11-09-0782.
14. Knoblach, B., Rachubinski, R.A. Phosphorylation-dependent activation of peroxisome proliferator protein PEX11 controls peroxisome abundance. *J Biol Chem*. 2010; 285: 6670–80. doi: 10.1074/jbc.M109.094805.
15. Thomas, A.S., Krikken, A.M., van der Klei, I.J., Williams, C.P. Phosphorylation of Pex11p does not regulate peroxisomal fission in the yeast *Hansenula polymorpha*. *Sci Rep*. 2015; 5: 11493. doi: 10.1038/srep11493.
16. van Roermund, C.W., Tabak, H.F., van Den Berg, M., Wanders, R.J., Hettema, E.H. Pex11p plays a primary role in medium-chain fatty acid oxidation, a process that affects peroxisome number and size in *Saccharomyces cerevisiae*. *J Cell Biol*. 2000; 150: 489–98. doi: 10.1083/jcb.150.3.489.
17. Mindthoff, S., Grunau, S., Steinfort, L.L., Girzalsky, W., Hiltunen, J.K., Erdmann, R., et al. Peroxisomal Pex11 is a pore-forming protein homologous to TRPM channels. *Biochim Biophys Acta*. 2016; 1863: 271–83. doi: 10.1016/j.bbamcr.2015.11.013.
18. Heymann, J.A.W., Hinshaw, J.E. Dynamins at a glance. *J Cell Sci.* 2009; 122: 3427–31. doi: 10.1242/jcs.051714.
19. Kuravi, K., Nagotu, S., Krikken, A.M., Sjollema, K., Deckers, M., Erdmann, R., et al. Dynamin-related proteins Vps1p and Dnm1p control peroxisome abundance in *Saccharomyces cerevisiae*. *J Cell Sci.* 2006; 119: 3994–4001. doi: 10.1242/jcs.03166.

20. Nagotu, S., Saraya, R., Otzen, M., Veenhuis, M., van der Klei, I.J. Peroxisome proliferation in *Hansenula polymorpha* requires Dnm1p which mediates fission but not *de novo* formation. *Biochim Biophys Acta*. 2008; 1783: 760–9. doi: 10.1016/j.bbamcr.2007.10.018.

21. Rosenberger, S., Connerth, M., Zellnig, G., Daum, G. Phosphatidylethanolamine synthesized by three different pathways is supplied to peroxisomes of the yeast *Saccharomyces cerevisiae*. *Biochim Biophys Acta*. 2009; 1791: 379–87. doi: 10.1016/j.bbalip.2009.01.015.

22. Wriessnegger, T., Gübitz, G., Leitner, E., Ingolic, E., Cregg, J., de la Cruz, B.J., et al. Lipid composition of peroxisomes from the yeast *Pichia pastoris* grown on different carbon sources. *Biochim Biophys Acta*. 2007; 1771: 455–61. doi: 10.1016/j.bbalip.2007.01.004.

23. Titorenko, V.I., Chan, H., Rachubinski, R.A. Fusion of small peroxisomal vesicles in vitro reconstructs an early step in the in vivo multistep peroxisome assembly pathway of *Yarrowia lipolytica*. *J Cell Biol*. 2000; 148: 29–44. doi: 10.1083/jcb.148.1.29.

24. Raychaudhuri, S., Prinz, W.A. Nonvesicular phospholipid transfer between peroxisomes and the endoplasmic reticulum. *Proc Natl Acad Sci U S A*. 2008; 105: 15785–90. doi: 10.1073/pnas.0808321105.

25. Prinz, W.A. Bridging the gap: membrane contact sites in signaling, metabolism, and organelle dynamics. *J Cell Biol*. 2014; 205: 759–69. doi: 10.1083/jcb.201401126.

26. Knoblach, B., Sun, X., Coquelle, N., Fagarasanu, A., Poirier, R.L., Rachubinski, R.A. An ER-peroxisome tether exerts peroxisome population control in yeast. *EMBO J*. 2013; 32: 2439–53. doi: 10.1038/emboj.2013.170.

27. David, C., Koch, J., Oeljeklaus, S., Laernsack, A., Melchior, S., Wiese, S., et al. A combined approach of quantitative interaction proteomics and live-cell imaging reveals a regulatory role for endoplasmic reticulum (ER) reticulon homology proteins in peroxisome biogenesis. *Mol Cell Proteomics MCP*. 2013; 12: 2408–25. doi: 10.1074/mcp.M112.017830.

28. Mast, F.D., Jamakhandi, A., Saleem, R.A., Dilworth, D.J., Rogers, R.S., Rachubinski, R.A., et al. Peroxins Pex30 and Pex29 Dynamically Associate with Reticulons to Regulate Peroxisome Biogenesis from the Endoplasmic Reticulum. *J Biol Chem*. 2016; 291: 15408–27. doi: 10.1074/jbc.M116.728154.

29. Costello, J.L., Castro, I.G., Hacker, C., Schrader, T.A., Metz, J., Zeuschner, D., et al. ACBD5 and VAPB mediate membrane associations between peroxisomes and the ER. *J Cell Biol*. 2017; 216: 331–42. doi: 10.1083/jcb.201607055.

30. Hua, R., Cheng, D., Coyaud, É., Freeman, S., Di Pietro, E., Wang, Y., et al. VAPs and ACBD5 tether peroxisomes to the ER for peroxisome maintenance and lipid homeostasis. *J Cell Biol*. 2017; 216: 367–77. doi: 10.1083/jcb.201608128.

31. Costello, J.L., Castro, I.G., Schrader, T.A., Islinger, M., Schrader, M. Peroxisomal ACBD4 interacts with VAPB and promotes ER-peroxisome associations. *Cell Cycle*. 2017; 16: 1039–45. doi: 10.1080/15384101.2017.1314422.

32. Cohen, Y., Klug, Y.A., Dimitrov, L., Erez, Z., Chuartzman, S.G., Elinger, D., et al. Peroxisomes are juxtaposed to strategic sites on mitochondria. *Mol Biosyst*. 2014; 10: 1742–8. doi: 10.1039/c4mb00001c.

33. Mattiazzi Ušaj, M., Brložnik, M., Kaferle, P., Žitnik, M., Wolinski, H., Leitner, F., et al. Genome-Wide Localization Study of Yeast Pex11 Identifies Peroxisome-Mitochondria Interactions through the ERMES Complex. *J Mol Biol*. 2015; 427: 2072–87. doi: 10.1016/j.jmb.2015.03.004.

34. Shai, N., Yifrach, E., van Roermund, C.W.T., Cohen, N., Bibi, C., IJlst, L., et al. Systematic mapping of contact sites reveals tethers and a function for the peroxisome-mitochondria contact. *Nat Commun*. 2018; 9: 1761. doi: 10.1038/s41467-018-03957-8.

35. Titorenko, V.I., Rachubinski, R.A. Mutants of the yeast *Yarrowia lipolytica* defective in protein exit from the endoplasmic reticulum are also defective in peroxisome biogenesis. *Mol Cell Biol*. 1998; 18: 2789–803. doi: 10.1128/mcb.18.5.2789.

36. Lam, S.K., Yoda, N., Schekman, R. A vesicle carrier that mediates peroxisome protein traffic from the endoplasmic reticulum. *Proc Natl Acad Sci U S A*. 2010; 107: 21523–8. doi: 10.1073/pnas.1013397107.

37. Agrawal, G., Joshi, S., Subramani, S. Cell-free sorting of peroxisomal membrane proteins from the endoplasmic reticulum. *Proc Natl Acad Sci U S A*. 2011; 108: 9113–8. doi: 10.1073/pnas.1018749108.

38. Kragt. A., Voorn-Brouwer, T., van den Berg, M., Distel, B. Endoplasmic reticulum-directed Pex3p routes to peroxisomes and restores peroxisome formation in a *Saccharomyces cerevisiae* pex3Delta strain. *J Biol Chem.* 2005; 280: 34350–7. doi: 10.1074/jbc.M505432200.

39. Fakieh, M.H., Drake, P.J.M., Lacey, J., Munck, J.M., Motley, A.M., Hettema, E.H. Intra-ER sorting of the peroxisomal membrane protein Pex3 relies on its luminal domain. *Biol Open*. 2013; 2: 829–37. doi: 10.1242/bio.20134788.

40. van der Zand, A., Braakman, I., Tabak, H.F. Peroxisomal membrane proteins insert into the endoplasmic reticulum. *Mol Biol Cell*. 2010; 21: 2057–65. doi: 10.1091/mbc.E10-02-0082.

41. Stroobants, A.K., Hettema, E.H., van den Berg, M., Tabak, H.F. Enlargement of the endoplasmic reticulum membrane in *Saccharomyces cerevisiae* is not necessarily linked to the unfolded protein response via Ire1p. *FEBS Lett*. 1999; 453: 210–4. DOI: 10.1016/s0014-5793(99)00721-8.

42. Matsuzaki, T., Fujiki, Y. The peroxisomal membrane protein import receptor Pex3p is directly transported to peroxisomes by a novel Pex19p- and Pex16p-dependent pathway. *J Cell Biol.* 2008; 183: 1275–86. doi: 10.1083/jcb.200806062.

43. Knoops, K., Manivannan, S., Cepinska, M.N., Krikken, A.M., Kram, A.M., Veenhuis, M., et al. Preperoxisomal vesicles can form in the absence of Pex3. *J Cell Biol*. 2014; 204: 659–68. doi: 10.1083/jcb.201310148.

44. Wróblewska, J.P., Cruz-Zaragoza, L.D., Yuan, W., Schummer, A., Chuartzman, S.G., de Boer R., et al. *Saccharomyces cerevisiae* cells lacking Pex3 contain membrane vesicles that harbor a subset of peroxisomal membrane proteins. *Biochim Biophys Acta Mol Cell Res*. 2017; 1864: 1656–67. doi: 10.1016/j.bbamcr.2017.05.021.

45. Joshi, A.S., Huang, X., Choudhary, V., Levine, T.P., Hu, J., Prinz, W.A. A family of membrane-shaping proteins at ER subdomains regulates pre-peroxisomal vesicle biogenesis. *J Cell Biol.* 2016; 215: 515–29. doi: 10.1083/jcb.201602064.

46. Joshi, A.S., Nebenfuehr, B., Choudhary, V., Satpute-Krishnan, P., Levine, T.P., Golden, A., et al. Lipid droplet and peroxisome biogenesis occur at the same ER subdomains. *Nat Commun*. 2018; 9: 2940. doi: 10.1038/s41467-018-05277-3.

47. Mast, F.D., Herricks, T., Strehler, K.M., Miller, L.R., Saleem, R.A., Rachubinski, R.A., et al. ESCRT-III is required for scissioning new peroxisomes from the endoplasmic reticulum. *J Cell Biol*. 2018; 217: 2087–102. doi: 10.1083/jcb.201706044.

48. Kim, P.K., Mullen, R.T., Schumann, U., Lippincott-Schwartz, J. The origin and maintenance of mammalian peroxisomes involves a *de novo* PEX16-dependent pathway from the ER. *J Cell Biol*. 2006; 173 :521–32. doi: 10.1083/jcb.200601036.

49. Yonekawa, S., Furuno, A., Baba, T., Fujiki, Y., Ogasawara, Y., Yamamoto, A., et al. Sec16B is involved in the endoplasmic reticulum export of the peroxisomal membrane biogenesis factor peroxin 16 (Pex16) in mammalian cells. *Proc Natl Acad Sci U S A*. 2011; 108: 12746–51. doi: 10.1073/pnas.1103283108.

50. Sugiura, A., Mattie, S., Prudent, J., McBride, H.M. Newly born peroxisomes are a hybrid of mitochondrial and ER-derived pre-peroxisomes. *Nature*. 2017; 542: 251–4. doi: 10.1038/nature21375.

51. Schueller, N., Holton, S.J., Fodor, K., Milewski, M., Konarev, P., Stanley, W.A., et al. The peroxisomal receptor Pex19p forms a helical mPTS recognition domain. *EMBO J*. 2010; 29: 2491–500. doi: 10.1038/emboj.2010.115.

52. Fang, Y., Morrell, J.C., Jones, J.M., Gould, S.J. PEX3 functions as a PEX19 docking factor in the import of class I peroxisomal membrane proteins. *J Cell Biol.* 2004; 164: 863–75. doi: 10.1083/jcb.200311131.

53. Sato, Y., Shibata, H., Nakatsu, T., Nakano, H., Kashiwayama, Y., Imanaka, T., et al. Structural basis for docking of peroxisomal membrane protein carrier Pex19p onto its receptor Pex3p. *EMBO J*. 2010; 29: 4083–93. doi: 10.1038/emboj.2010.293.

54. Halbach, A., Rucktäschel, R., Rottensteiner, H., Erdmann, R. The N-domain of Pex22p can functionally replace the Pex3p N-domain in targeting and peroxisome formation. *J Biol Chem*. 2009; 284: 3906–16. doi: 10.1074/jbc.M806950200.

55. van der Zand, A., Gent, J., Braakman, I., Tabak, H.F. Biochemically distinct vesicles from the endoplasmic reticulum fuse to form peroxisomes. *Cell*. 2012; 149: 397–409. doi: 10.1016/j.cell.2012.01.054.

56. South, S.T., Baumgart, E., Gould, S.J. Inactivation of the endoplasmic reticulum protein translocation factor, Sec61p, or its homolog, Ssh1p, does not affect peroxisome biogenesis. *Proc Natl Acad Sci U S A*. 2001; 98: 12027–31. doi: 10.1073/pnas.221289498.

57. Fagarasanu, M., Fagarasanu, A., Tam, Y.Y.C., Aitchison, J.D., Rachubinski, R.A. Inp1p is a peroxisomal membrane protein required for peroxisome inheritance in *Saccharomyces cerevisiae. J Cell Biol*. 2005; 169: 765–75. doi: 10.1083/jcb.200503083.

58. Krikken, A.M., Veenhuis, M., van der Klei, I.J. *Hansenula polymorpha pex11* cells are affected in peroxisome retention. *FEBS J*. 2009; 276: 1429–39. doi: 10.1111/j.1742-4658.2009.06883.x.

59. Hoepfner, D., van den Berg, M., Philippsen, P., Tabak, H.F., Hettema, E.H. A role for Vps1p, actin, and the Myo2p motor in peroxisome abundance and inheritance in *Saccharomyces cerevisiae. J Cell Biol.* 2001; 155: 979–90. doi: 10.1083/jcb.200107028.

60. Fagarasanu, A., Mast, F.D., Knoblach, B., Jin, Y., Brunner, M.J., Logan, M.R., et al. Myosin-driven peroxisome partitioning in *S. cerevisiae. J Cell Biol*. 2009; 186: 541–54. doi: 10.1083/jcb.200904050.

61. Motley, A.M., Hettema, E.H. Yeast peroxisomes multiply by growth and division. *J Cell Biol*. 2007; 178: 399–410. doi: 10.1083/jcb.200702167.

62. Otzen, M., Rucktäschel, R., Thoms, S., Emmrich, K., Krikken, A.M., Erdmann, R., et al. Pex19p contributes to peroxisome inheritance in the association of peroxisomes to Myo2p. *Traffic Cph Den.* 2012; 13: 947–59. doi: 10.1111/j.1600-0854.2012.01364.x.

63. Salogiannis, J., Egan, M.J., Reck-Peterson, S.L. Peroxisomes move by hitchhiking on early endosomes using the novel linker protein PxdA. *J Cell Biol.* 2016; 212: 289–96. doi: 10.1083/jcb.201512020.

64. Huybrechts, S.J., Van Veldhoven, P.P., Brees, C., Mannaerts, G.P., Los, G.V., Fransen, M. Peroxisome dynamics in cultured mammalian cells. *Traffic Cph Den*. 2009; 10: 1722–33. doi: 10.1111/j.1600-0854.2009.00970.x.

65. Kumar, S., de Boer, R., van der Klei, I.J. Yeast cells contain a heterogeneous population of peroxisomes that segregate asymmetrically during cell division. *J Cell Sci*. 2018; 131. doi: 10.1242/jcs.207522.

66. Liu, X., Ma, C., Subramani, S. Recent advances in peroxisomal matrix protein import. *Curr Opin Cell Biol*. 2012; 24: 484 – 489. doi: 10.1016/j.ceb.2012.05.003.

67. Erdmann, R., Blobel, G. Giant peroxisomes in oleic acid-induced *Saccharomyces cerevisiae* lacking the peroxisomal membrane protein Pmp27p. *J Cell Biol.* 1995; 128: 509–523. doi: 10.1083/jcb.128.4.509.

68. Motley, A.M., Ward, G.P., Hettema, E.H. Dnm1p-dependent peroxisome fission requires Caf4p, Mdv1p and Fis1p. *J Cell Sci.* 2008; 121: 1633–40. doi: 10.1242/jcs.026344.

69. Nagotu, S., Krikken, A.M., Otzen, M., Kiel, J.A., Veenhuis, M., van der Klei, I.J. Peroxisome fission in *Hansenula polymorpha* requires Mdv1 and Fis1, two proteins also involved in mitochondrial fission. *Traffic*. 2008; 9: 1471-84. doi: 10.1111/j.1600-0854.2008.00772.x.

70. Haan, G.J., Baerends, R.J., Krikken, A.M., Otzen, M., Veenhuis, M., van der Klei, I.J. Reassembly of peroxisomes in *Hansenula polymorpha pex3* cells on reintroduction of Pex3p involves the nuclear envelope. *FEMS Yeast Res*. 2006; 6: 186-94. doi: 10.1111/j.1567-1364.2006.00037.x.