Origin and growth of peroxisomes in yeast

Yuan, Wei

Summary

The study of peroxisomes began in the 1950's with the electron microscopic discovery by Rhodin that mouse kidney cells contain single membrane bound structures with diameters between 0.1-1.0 μm, which were initially named microbodies (Rhodin et al., 1954). Later in the 1960's, the term 'microbodies' was renamed to peroxisomes, because several hydrogen peroxide producing oxidases were shown to be present in these organelles (De Duve et al., 1966; 1969).

Now, we know that peroxisomes are very common cell organelles that occur in almost all eukaryotic cells and display a large variety of metabolic and non-metabolic functions. Their size, morphology and function strongly depend on the cells in which they occur. In man, peroxisomes play key roles in the oxidation of very long chain fatty acids and plasmalogen biosynthesis. As a consequence defects in peroxisome formation result in severe, inherited diseases (called Peroxisome Biogenesis Disorders (PBDs)) some of which are lethal. In germinating plant oil seeds, enzymes of the β-oxidation of fatty acids and key enzymes of the glyoxylate cycle are localized in peroxisomes (also called glyoxysomes). In yeast, peroxisomes are crucial for the metabolism of several unusual carbon and nitrogen sources (i.e. methanol, oleic acid, alkanes, primary amines, purines). In filamentous fungi, peroxisomes are involved in the formation of a variety of secondary metabolites, such as antibiotics. Moreover, in some filamentous fungi peroxisomes also play a role in development and differentiation, whereas specialized peroxisomes, called Woronin bodies, play a structural role in plugging septal pores (Jedd.,2011).

There is a long debate on the origin of peroxisomes. These organelles could either be formed by fission of pre-existing peroxisomes, or *de novo* from the endoplasmic reticulum (ER). Several proteins required for peroxisome fission

have been identified (e.g. Pex11, Dnm1, Vps1). The *de novo* model is based on the assumption that peroxisomal membrane remnants are absent when genes encoding the peroxins Pex3 or Pex19 are deleted.

The *de novo* model predicts that all PMPs are first sorted to the ER in WT cells, and are subsequently enclosed in two different types of ER-derived vesicles, that contain both one half of the peroxisomal translocon. According to this model, Pex3 and Pex19 are required for the exit of PMPs from the ER. Subsequently, these two types of vesicles fuse with each other dependent of Pex1/Pex6. Hence, in cells lacking Pex1 and Pex6, functional peroxisomal translocons cannot assemble because they are physically separated.

However, this model was recently challenged by the studies that showed that a subset of PMPs (Pex13, Pex14, Pex8) are present in preperoxisomal vesicles in *Hansenula polymorpha pex3* cells indicating that Pex3 is not required for the exit of at least Pex8, Pex13 and Pex14 to preperoxisomal vesicles. Recent studies also revealed that yeast Pex1 and Pex6 are not involved in the fusion of ER-derived vesicles, but play a role in peroxisomal matrix protein import as initially suggested.

Upon formation of a new peroxisome by either fission or *de novo* synthesis, the nascent organelles growth into a mature one. Growth of peroxisomes not only involves the incorporation of peroxisomal membrane and matrix proteins, but also of membrane lipids. But, yeast peroxisomes lack of a membrane lipid synthesizing machinery. Therefore, peroxisomes must obtain their membrane lipids from other sources, which may occur by either non-vesicular pathways or vesicular pathways or a combination of these two. The non-vesicular model predicts that lipids transfer directly from other cellular membranes to peroxisomes at membrane contact sites. In the vesicular pathway ER-derived vesicles most likely fuse with pre-existing

peroxisomes contributing to lipid transfer.

The aim of this thesis is to address the origin of peroxisomes, as well as the molecular mechanisms of organellar membrane growth in yeast.

Chapter 1 highlights our current knowledge on the origin of yeast peroxisomes, focusing on the phenotypes of various peroxisome deficient mutants. For some yeast *PEX* deletion strains, different mutant phenotypes have been reported. However, we propose that this may not be correct but is due to the fact that different marker proteins, experimental procedures and species were used. We discuss the current main models of peroxisome formation: peroxisome fission and *de novo* synthesis from the ER. In addition, this chapter provides an overview on the molecular mechanisms involved in the biogenesis of the peroxisomal membrane, which involves sorting of peroxisomal membrane proteins and membrane lipids.

The research described in **Chapter 2** shows that *S. cerevisiae pex3* cells contain vesicular peroxisomal membrane structures, which contradicts the earlier view that peroxisomal membrane structures are fully absent in *S. cerevisiae pex3* cells. In contrast to the membrane structures in *H. polymorpha pex3* cells, in *S. cerevisiae pex3* cells the vesicles are stable and not degraded by autophagy. Probably, this is due to the fact that Pex3 is required for pexophagy in *S. cerevisiae*. The vesicular structures in *S. cerevisiae pex3* cells harbor the PMP Pex14 and the matrix protein Pex8, indicating that the current *de novo* model is not valid because this model predicts that these peroxins accumulate at the ER in *pex3* cells. Relative to WT controls, the levels of Pex10, Pex11, Pex13 and Ant1 were strongly reduced in *pex3 atg1* cells, suggesting that the sorting of these PMPs is blocked. Where GFP fusion proteins of Pex10, Pex13 and Ant1 were below the limit of detection by fluorescence microscopy, Pex11-GFP was mislocalized to mitochondria. To test whether the vesicular peroxisomal

structures in *S. cerevisiae pex3* can develop into mature peroxisomes upon reintroduction of Pex3, we introduced an auxin-inducible degron system to modulate the levels of Pex3. Using time-lapse videos, we showed that Pex14 containing vesicles in cells lacking Pex3 can mature into peroxisomes upon reintroduction of Pex3, suggesting that peroxisomes in *S. cerevisiae pex3* cells are not formed *de novo* from the ER.

In **Chapter 3** we showed that Vps13 plays an important role in peroxisome biogenesis in *H. polymorpha pex11* and *pex23* mutant cells, because cells of *pex11, pex23* and *vps13* single deletion strains contain functional peroxisomes, whereas these are absent in *pex11 vps13* and *pex23 vps13* double deletion strains. Vps13 was previously shown to regulate two vacuolar membrane contact sites: the Nuclear Vacuole Junction (NVJ) and vCLAMP (vacuole and mitochondrial patch). Based on our results we speculate that Vps13 may play a role in peroxisome vacuole contact sites as well.

Based on earlier studies in *S. cerevisiae*, we propose that *H. polymorpha* Pex23 is involved in the formation of ER-peroxisome membrane contact sites (EPCONS). Indeed, FM analysis revealed that Pex23 colocalizes with both ER and peroxisomal marker proteins. Most likely Pex11 also plays a function in the formation of a peroxisomal MCS. We hypothesize that different redundant peroxisomal MCSs exist that are required for growth of the peroxisomal membrane. This explains why only a strong effect on peroxisome formation is observed when more than one MCS is defective at the same time (i.e. in *pex11 vps13* and *pex23 vps13* cells).

This hypothesis is supported by detailed microscopy analyses, which revealed that *pex11 vps13* cells harbor vesicles that contain a minor portion of the peroxisomal matrix proteins and in addition contain all tested PMPs (Pex3, Pex8, Pex10, Pex11, Pex14, Pmp47). Hence, these structures most likely represent small peroxisomes that have the ability to import

matrix and membrane proteins, but are not able to grow due to a defect in the peroxisomal membrane expansion machinery.

In addition, we showed that *pex11 vps13* and *pex23 vps13* regain functional peroxisomes by introduction of an artificial ER-peroxisome tethering protein. This suggests that in both *pex23* and *pex11* EPCONS may be defective, which can be partially repressed by the ER-peroxisome tethering protein. Finally, analysis of *S. cerevisiae pex11 vps13* cells revealed that this double mutant is also peroxisome deficient, indicating that the phenotype we observed in *H. polymorpha pex11 vps13* is conserved in different yeast species.

In **Chapter 4** we further analyze peroxisomal membrane contact sites. We show that in *H. polymorpha* at peroxisome repressing growth conditions (glucose) peroxisomes are only associated with the ER, whereas upon a shift of cells to peroxisome inducing conditions (methanol) both the vacuole and the ER are in close contact with peroxisomes, indicating that these organelles may be important for peroxisome biogenesis.

Because Vps13 regulates vCLAMP, we also analyzed the role of the vCLAMP components Ypt7, Vam7 and Vps39 in peroxisome biogenesis. Interestingly, these proteins partially colocalize with peroxisome marker proteins at peroxisome inducing conditions. Interestingly, the absence of either vCLAMP proteins or deletion of components of the putative ER-peroxisome MCSs (Pex11, Pex23) results in a mild or no peroxisomal phenotype, whereas disruption both MCSs (e.g. in *pex11 ypt7* or *pex23 ypt7*) results in a severe defect in peroxisome formation, similar as observed for *pex11 vps13* and *pex23 vps13* (Chapter 3).

Like in *pex11 vps13*, also in *pex11 ypt7* cells, the peroxisome biogenesis defect could be largely suppressed by the introduction of an artificial ER-peroxisome tethering.

It is worth noting that small peroxisomes are still present in

pex11 and *pex23* strains lacking v*CLAMP* proteins. Obviously transport of membrane lipids still occurs. Possibly, mitochondrial MCSs may contribute to lipid transport or the vacuolar and/or ER MCSs are still partially functioning. Alternatively, these organelles may obtain lipids via vesicle mediated transport or form *de novo* from the ER. Further studies are required to further elucidate these important questions.

References

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