Peroxisome biogenesis and maintenance in yeast

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Summary

Eukaryota, Bacteria and Archaea represent three domains of life. Eukaryotic organisms are distinguished by the presence of complex compartments, called organelles, that are separated from the rest of the cell by biological membranes. Organelles are characterized by their high specialization that provides optimal conditions for different processes.

One class of organelles includes peroxisomes. These single membrane bound structures were identified in 1954 by Rhodin [1]. However, their first biochemical characterization as a novel cellular compartment was carried out by de Duve and his team years later [2,3]. Peroxisomes fulfill various functions. Conserved ones include β-oxidation of fatty acids and detoxification of hydrogen peroxide. Peroxisomes are essential in humans. The importance of peroxisomes is evident from severe disorders caused by the mutations in *PEX* genes encoding proteins (peroxins) that play key roles in peroxisome biogenesis.

Yeast provide an excellent system for studies of peroxisome formation since peroxisomes are not essential for viability in these organisms. What is more, peroxisome formation and degradation can be easily induced by manipulation of the yeast growth conditions.

Peroxisomes can be formed in two different ways. Similar to mitochondria, they can form from pre-existing peroxisomes by their division. On the other hand, peroxisomes can also form *de novo* from the ER. There is evidence suggesting that fission of peroxisomes is the prevailing way of peroxisome proliferation in yeast wild type cells. The contribution of these two modes of peroxisome biogenesis to the total population of peroxisomes in WT cells is a subject of an ongoing debate.

In **Chapter 1** we summarized the current knowledge on peroxisome biology. We focused mainly on the recent advances revealing mechanisms of peroxisome fission, *de novo* formation, inheritance and peroxisomal membrane expansion.

Chapter 2 discusses our research on the pre-peroxisomal vesicles (PPVs) that were discovered in *S. cerevisiae pex3* cells through detailed microscopic studies. It had been proposed that peroxisomal membrane proteins (PMPs) traffic to peroxisomal membranes via the ER and that Pex3 is implicated in their exit from this compartment, in form of vesicles. According to this model, it is expected that peroxisomal membranes are fully absent in *pex3* cells and PMPs accumulate at the ER. In contrast, we showed that *S. cerevisiae* cells lacking Pex3 are not completely devoid of peroxisomal membranes. The vesicular structures were previously described in *H. polymorpha*

[4] where they were susceptible for autophagic degradation, as opposed to the vesicles in *S. cerevisiae*. We showed that out of nineteen PMPs that were tested, none was found to localize at the ER in the absence of Pex3. This observation is not in line with the model suggesting that all PMPs first sort to the ER during *de novo* biogenesis of peroxisomes and accumulate there in the absence of Pex3. Instead, we showed that a substantial group of tested PMPs localize to membrane vesicles, where they correctly assemble with each other, because a structure resembling the PTS1 protein import pore, consisting of Pex14, Pex13, Pex17 and Pex5, is formed. Some of the PMPs mislocalized to other cellular compartments, whereas the levels of some PMPs was extremely low in the absence of Pex3. This suggests their dependence on Pex3 for insertion into the peroxisomal membrane. Our findings challenge the view that the Pex3/Pex19 machinery is essential for recruiting all newly synthesized PMPs to the peroxisomal membrane and points out to the existence of other pathways of PMP delivery. This suggests that there is still a lot to discover in order to fully understand the molecular mechanisms of peroxisomal membrane biogenesis.

Following that thought, we analyzed peroxisomal membrane vesicles in a more detailed study. In **Chapter 3** we presented work that aimed to identify protein components of PPVs in yeast *pex3* cells, as well as proteins required for the formation of these structures. Automated mating, sporulation and mutant selection approaches were used in order to construct two yeast libraries, tailor-made for studies on the membrane vesicles present in *pex3 atg1* mutant cells. The first library consisted of *pex3 atg1* double mutants expressing Pex14-mCherry, as a marker of peroxisomal membrane structures, together with proteins N-terminally tagged with GFP, covering approximately 2000 proteins of the *S. cerevisiae* proteome. High throughput fluorescence microscopy (HT-FM) was employed to image the library with the aim to detect co-localization of the two fluorescence signals. As a result, we found a number of proteins that possibly (partially) localize to PPVs, as evident from their co-localization with Pex14-mCherry. Apart from peroxins, we also found proteins typical to other cellular compartments. This suggests that peroxisomal vesicles may derive their membrane from a different organelle. Another plausible explanation is that peroxisomes share the biogenesis site in the cell with other organelles. In the second approach we aimed to construct a library made up of triple mutants that contain deletions of *PEX3*, *ATG1* and an additional third gene, covering all non-essential genes of *S. cerevisiae*. Analysis of this library did not result in the identification of triple mutants lacking Pex14-mGFP spots suggesting that the biogenesis of PPVs is a complex process involving the function of many protein players. However, some of the putative triple mutants still contained the *PEX3* gene due to selection conditions that turned out to be excessively mild. This and other pitfalls of the experimental approaches used are discussed.

Research of organelle contacts has been advancing very rapidly in the recent years. Occurrence of membrane contact sites (MCSs) is a common phenomenon and their potential involvement in organelle biology should not be neglected. For that reason, in **Chapter 4** we focused on the function of a vacuolar protein - Vac8, which was detected in peroxisomal fractions of *S. cerevisiae* [5] and *H. polymorpha* [6]. Vac8 has been extensively studied in *S. cerevisiae,* where it is required for vacuole inheritance and fusion. *Sc*Vac8 is also essential for the formation of MCSs designated nucleus-vacuole junctions (NVJs), together with its binding partner on the nuclear envelope - Nvj1 [7].

In silico analysis of the *H. polymorpha* genome to find homologs of *S. cerevisiae* NVJ proteins identified all homologs of NVJ-related proteins, except Nvj1. This indicates that the composition of NVJs among species is not conserved. According to our results, *Hp*Vac8 is essential for NVJ formation, as no close contact between the nucleus and vacuole could be observed in the *vac8* mutant. We also showed that, similarly to *S. cerevisiae*, *Hp*Vac8 is important for vacuole inheritance. However, the absence of *Hp*Vac8 had no effect on vacuole-vacuole fusion. Our data imply that *Hp*Vac8 is unlikely to be involved in peroxisome biology or function. This conclusion is based on the fact that neither deletion nor overpexpression of *VAC8* had an effect on peroxisome abundance. Therefore, the reason for the presence of Vac8 in peroxisomal fractions is not obvious and remains to be unraveled.

Although *de novo* biogenesis of peroxisomes is an attractive model for cells temporarily devoid of these organelles, growth and fission is the prevailing mode of peroxisome formation in WT yeast cells. In the final chapter of this thesis (**Chapter 5**), we focused on modes of peroxisome acquisition in buds of yeast mutants defected in fission and/or inheritance. We described our discovery that fission of peroxisomes in *H. polymorpha* can proceed without an important component of the organelle fission machinery, namely Pex11. Detailed live cell imaging revealed that in many of the *pex11* mutant cells residual fission still occurs and is followed by transfer of a small peroxisome to nascent buds. We noticed that only by introduction of simultaneous defects in fission and inheritance, by deleting *PEX11* and *INP2*, fission is no longer observed. As a result, newly formed buds of the *pex11 inp2* mutant are initially devoid of peroxisomes, which are most likely synthesized *de novo* at later stages of bud formation. *De novo* biogenesis is a slow process compared to peroxisome maturation from an inherited membrane. However, it is most likely important to maintain a peroxisome population at conditions where their function is required for growth. We also show that inheritance of peroxisomes in *H. polymorpha* can occur independently of Inp2, via a mechanism that is yet to be revealed.

Outlook

Regardless of the considerable advances in understanding of molecular mechanisms involved in peroxisome matrix protein import [8], there is still a large gap in our knowledge regarding peroxisome membrane formation.

The identification of PPVs in yeast *pex3* mutants supports the view that Pex3 is not crucial for biogenesis of peroxisomal membranes. The importance of Pex3 manifests itself in the process of maturation of these vesicles into functional peroxisomes, which do not form in the absence of this peroxin.

The challenge for future research, aiming to unravel the scientific enigma of peroxisome biogenesis, lies in finding novel routes of PMPs targeting to peroxisomal membranes, that are not dependent on Pex3. That will shed more light on the mechanisms of peroxisomal vesicle formation, bringing us much closer to understanding of the biogenesis of peroxisomes themselves. More detailed studies of interesting strains found through the HT screens presented in this thesis may lead to selection of promising candidates that play a key role in biogenesis of peroxisomes. Moreover, additional HT screens may be useful in identifying proteins crucial in this process.

Our data showed that in yeast cells defective in fission and inheritance cells are devoid of peroxisomes and form these organelles *de novo*. The important question that follows is whether the *de novo* formation of functional peroxisomes goes through an intermediate step that involves formation of PPVs, the same as present in *pex3* mutant cells. In order to reveal that it would be necessary to perform a detailed microscopy study of the *pex11 inp2* mutant in search of peroxisomal vesicles. Without any doubt, further studies are essential in order to provide the long-awaited answers elucidating the mechanisms behind peroxisome biogenesis.

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