Origin and growth of peroxisomes in yeast

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Origin and growth of peroxisomes in yeast

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Preface

At October 1st, 2011, it was a special day for all the Chinese to celebrate China to become an independent country. To me, it was also a big day. With greatly happiness, I arrived in Netherlands and started my PhD with exploration in my life.

I would say that the experience in the Netherlands will be unforgettable days forever in my whole life. I would like to take this chance to express my gratitude to the following people.

First, I would like to thank Prof. dr. I.J. van der Klei to accept me to be a member of Molecular Cell Biology group. I am very grateful for her patience, motivation, and support. When I joined in our group, it is a difficult environment for me to adapt to as I have difficulty in communicating with the other people by English. She was always patient to listen my crappy English and encouraged me to communicate more with our colleagues. Her passion and attitude to science also infected me deeply throughout my whole PhD. What she behaved in academy is a good treasure to me for developing my career in the future.

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My project is very new in the peroxisome field. Too many things need to be explored and it is impossible for me to finish

everything by myself. I would like to special thank Arman for his assistance in finishing a part of my projects and the so called ‘paper work’, and Rinse, Anita for the help in electron microscopy. Without them, I would not be able to achieve it.

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Aim and outline

Hallmark of eukaryotic cells is the compartmentalization of various functions into different membrane bound structures, the so called organelles. Of these, peroxisomes represent a special class of organelles because they participate in an unprecedented range of functions.

The importance of peroxisomes is highlighted by the severe inherited human disorders such as Zellweger syndrome, which are caused by peroxisome dysfunction. Until now, over 30 *PEX* genes, encoding peroxins, have been identified as being required for peroxisome formation. Mutations in *PEX* genes are not lethal in yeast, which renders these organisms ideal models to study the molecular mechanisms involved in peroxisome biogenesis.

For decades, the origin of peroxisomes is debated. Two different modes of peroxisome formation have been documented, namely fission from pre-existing organelles, and *de novo* formation of peroxisomes from the endoplasmic reticulum (ER). The first model is among others based on the identification of proteins that are essential for peroxisome fission (i.e. Dnm1, Vps1, Pex11). The most recent ER based model predicts that all peroxisomal membrane proteins (PMPs) are first sorted to the ER and subsequently end up in two types of ER-derived vesicles, a process that requires the peroxins Pex3 and Pex19. Subsequent heterotypical vesicle fusion, which depends on Pex1 and Pex6, leads to the formation of nascent peroxisomes. However, this latter model has recently been challenged by two independent studies that indicate that Pex1 and Pex6 are not involved in vesicle fusion. Also, most likely not all PMPs sort via the ER to peroxisomes. Instead, many if not most may directly insert into the peroxisomal membrane upon their synthesis in the cytosol.

Like sorting of PMPs also the transport pathways of

peroxisomal membrane lipids are still unresolved. According to the *de novo* peroxisome formation model, peroxisomes may receive membrane lipids from ER-derived vesicles. However, evidence for non-vesicular lipid transport pathways to peroxisomes has been presented as well.

The aim of this thesis is to understand the formation of peroxisomes, both regarding their origin and the subsequent expansion of the organellar membrane.

Chapter 1 discusses the recent advances in peroxisome formation, including PMP sorting and lipid incorporation.

In Chapter 2 we demonstrate that, akin to what was previously shown for *Hansenula polymorpha pex3* cells, peroxisomal membrane remnant structures are present in *Saccharomyces cerevisiae pex3* cells. Our data revealed that these structures in *S. cerevisiae pex3* are relatively stable and harbor the peroxisomal receptor docking protein Pex14 in conjunction with the matrix protein Pex8, challenging the view that all PMPs sort to the ER and exit this membrane by a Pex3-dependent process. Moreover, the peroxisome remnant structures could mature into functional peroxisomes upon reintroduction of Pex3, suggesting that under these conditions peroxisomes are not formed *de novo* from the ER.

Chapter 3 describes a novel role for Vps13, originally described to be essential for vacuolar protein sorting (Vps). This work is based on the observation that deletion of several *PEX* genes does not lead to peroxisome deficiency, but rather to aberrant peroxisome morphologies i.e. reduction of organelle numbers and/or size. Examples of such genes are *PEX11* and *PEX23*. We reasoned that these genes may have redundant functions with other genes involved in peroxisome formation. To test this hypothesis, we performed transposon mutagenesis of *H.*

polymorpha pex11 cells to search for these genes. This screening identified Vps13, a regulator of mitochondrial-vacuole and nuclear-vacuole membrane contact sites. Deletion of both *VPS13* and *PEX11* resulted in severe mislocalization of peroxisomal matrix proteins, which was not observed in *VSP13* or *PEX11* single deletion strains. Further analysis revealed that peroxisomal membrane structures are present in *H. polymorpha pex11 vps13* cells, which contained all PMPs tested (Pex3, Pex8, Pex10, Pex13, Pex14 and Pmp47), indicating that the targeting of PMPs to peroxisomal membranes is not defective in these cells.

Deletion of both *VPS13* and *PEX23*, a gene important for the formation of ER-peroxisome membrane contact sites (EPCONS) resulted in a similar phenotype as observed for *pex11 vps13* cells.

The small peroxisomal structures present in *pex11 vps13* cells were able to import minor amounts of matrix proteins, but failed to grow, suggesting that the membranes were unable to expand. Indeed, the peroxisome deficient phenotype of *pex11 vps13* and *pex23 vps13* cells could be largely suppressed upon artificially tethering the peroxisomal membrane structures to the ER.

Taking together, these findings suggest that Vps13 is important to compensate for a defect in MCSs that occurs in *pex11* and *pex23* cells, which are required for peroxisomal membrane expansion.

Vps13 has been implicated in the regulation of nuclear vacuole junctions (NVJs) and vCLAMP, a membrane contact site between vacuoles and mitochondria. As *H. polymorpha pex11 vps13* and *pex23 vps13* are peroxisome deficient, we subsequently tested whether vCLAMP proteins are also important for peroxisome biogenesis (Chapter 4).

At the morphological level, distinct membrane contacts between peroxisomes and vacuoles were very evident in

wild-type *H. polymorpha* cells grown at peroxisome proliferating conditions. Our data furthermore showed that components of vCLAMP (Ypt7, Vam7, Vps39) partially co-localize with peroxisomes. However, deletion of *YPT7*, *VAM7* or *VPS39* did not significantly affect peroxisome biogenesis.

Because deletion of *VPS13* in *pex11* or *pex23* cells (Chapter 3) caused peroxisome deficiency, we subsequently tested whether double deletion of *PEX11* or *PEX23* together with genes encoding vCLAMP proteins, caused peroxisome deficiency (i.e. *pex11 ypt7*, *pex11 vam7*, *pex11 vps39, pex23 ypt7, pex23 vps39).* Indeed, in all these double mutants the bulk of the peroxisomal matrix proteins was mislocalized to the cytosol. Detailed analysis of *pex11 ypt7* cells revealed that they contained small peroxisomes harboring all PMPs tested (i.e. Pex3, Pex8, Pex10, Pex13, Pex14, Pmp47), but most likely failed to grow in size. The peroxisomal phenotype of *pex11 ypt7* could be complemented by artificially tethering peroxisomes to the ER. Our findings suggest that peroxisome-vacuole contact sites together with EPCONS contribute to the membrane lipid supply to growing peroxisomes