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To my loving mom and the world-changing friends I made over the course of my Ph.D.
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Aim and outline

Peroxisomes belong to an important class of sub-cellular organelles present in essentially all eukaryotes. These highly dynamic organelles are involved in a wide range of functions which depend on the organism, cell type, developmental stage as well as internal and external cues.

PEX genes encode peroxins that are responsible for peroxisome formation. Mutations in those genes in human lead to severe disorders, often lethal at very early developmental stages. Because yeast *pex* mutants are viable, they provide an ideal system for studies of the molecular bases of peroxisome biogenesis.

Numerous studies addressing peroxisome biogenesis suggest two ways of peroxisome formation. The first one proposes growth and division of the pre-existing peroxisomes while the second one indicates that peroxisomes form *de novo* with an engagement of the endoplasmic reticulum (ER). Recent studies in yeast have led to the discovery of pre-peroxisomal vesicles (PPVs), which may represent early stages of peroxisomes in the *de novo* formation pathway. The aim of this thesis is to obtain further insights into this pathway.

Chapter 1 provides an overview of the current knowledge on peroxisome formation and inheritance in yeast.

In **Chapter 2** we show that peroxisomal membrane vesicles are present in an *S. cerevisiae pex3* mutant, as was previously demonstrated for *H. polymorpha pex3* cells. This finding counters the generally accepted view that cells lacking Pex3 are devoid of any peroxisomal membrane structures. At the vesicular structures a subset of peroxisomal membrane proteins (PMPs) (Pex14, Pex13, Pex17 and Pex5) assemble into a complex similar to the PTS1 protein translocation pore of WT yeast cells. Using a combination of microscopy and biochemical approaches, we show that the identified membrane vesicles do not represent a specialized region of the ER. Our results challenge the model proposing that all PMPs are first sorted to the ER and subsequently exit that compartment in the Pex3-depenent manner.

In **Chapter 3** we addressed the origin and protein composition of the peroxisomal membrane vesicles in *S. cerevisiae pex3* cells using two genetic screens that were based on automated mating, sporulation and mutant selection approaches combined with automated fluorescence microscopy. One of these screens, aiming to determine the protein composition of the peroxisomal vesicles, resulted in a list of proteins that co-localized with the peroxisomal vesicle marker protein

Pex14. We failed to identify proteins crucial for peroxisomal vesicle formation. Some of the risks associated with high-throughput approaches are discussed.

Our co-localization screen identified Nvj2, a protein of nucleus-vacuole junctions (NVJs), as a possible candidate protein associated with pre-peroxisomal vesicles in *S. cerevisiae pex3* cells (**Chapter 3**). Vac8, another NVJ protein, was identified in two independent organelle proteomics studies. **Chapter 4** describes studies aiming to elucidate whether Vac8 plays a role in peroxisome biogenesis in *H. polymorpha.* First we showed that *H. polymorpha* Vac8 is required for the formation of NVJs and vacuole inheritance, like in *S. cerevisiae*. However, *Hp*Vac8 is not required for vacuole fusion. The composition of the *H. polymorpha* NVJ differs from the one in *S. cerevisiae,* because of the absence of Nvj1, which is the second essential component for the formation of NVJs in baker's yeast. We were unable to detect any peroxisomal defect in *H. polymorpha* cells lacking Vac8, indicating that this protein most likely is not important in peroxisome biology.

Some reports suggest that peroxisomes are formed *de novo* from the ER in WT yeast cells. However, other studies indicate that peroxisomes predominantly multiply by fission and are carefully segregated over mother and bud during yeast budding. In **Chapter 5** we describe the consequences of impaired peroxisome fission and inheritance on the peroxisome population in *H. polymorpha*. Detailed fluorescence microscopy analysis revealed that peroxisome proliferation and inheritance are completely blocked in a *pex11 inp2* double deletion strain, because peroxisomes could not be detected in newly formed buds of this double mutant. At later stages, however, these structures could be identified, implying that the buds acquire them *de novo.* This study suggests that in *H. polymorpha de novo* peroxisome formation can occur, but serves only as a rescue mechanism for the formation of peroxisomes in mutant cells that lack these organelles.