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Peroxisome division and inheritance in yeast

Kuravi, Venkata Kasinath

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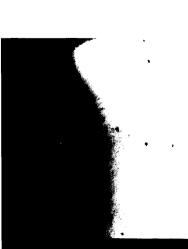nt on PEX5. *Mol.Biol.Cell* **16**:

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Summary

The cell is the basic unit of life. Based on their structure and composition, cells can be classified in two major groups, the prokaryotes (bacteria, Archaea) and the eukaryotes. A major morphological difference between the two is that eukaryotic cells harbour membrane-bound compartments (organelles) such as mitochondria, the endoplasmic reticulum, vacuoles, peroxisomes etc., while these compartments are absent in prokarytes. Each organelle is specialized to perform distinct tasks. For example, in the nucleus the genetic information (the DNA) is stored, mitochondria are responsible for energy generation from food and vacuoles act as sites of degradation and recycling.

Microbodies (peroxisomes, glyoxysomes, glycosomes) are functioning in a wide range of processes, which largely depend on the cell type and organism in which they occur. A common function of peroxisomes is the conversion of hydrogen peroxide into water and oxygen. In addition, the oxidation of fatty acids is a general peroxisome function. In man, peroxisomes are involved in a large range of metabolic processes. Main functions most likely are the catabolism of very long chain fatty acids (the so called β-oxidation), which are part of our diet, and the biosynthesis of ether lipids. The peroxisomal metabolic processes are very important, which is illustrated by the fact that inherited defects in the biogenesis or function of peroxisomes in man cause severe diseases that may be lethal.

In plant, two kinds of peroxisomes exist - peroxisomes and glyoxysomes. In green leaves peroxisomes function in photorespiration. Glyoxysomes occur in germinating oil bearing seeds. During seed germination, these fatty acids are catabolized by the β-oxidation system, resulting in C_2- compounds that are further metabolized by glyoxysomal enzymes.

In the filamentous fungus *Penicillium chrysogenum* microbodies host enzymes that are involved in the last steps of penicillin biosynthesis.

In yeast, the abundance of peroxisomes is highly depending on environmental conditions (i.e. the carbon and nitrogen sources used for growth). When yeast cells are grown on glucose as sole carbon source generally only one or a few peroxisomes are present per cell. Peroxisomes are not abundant in these cells, because peroxisomal enzymes are not involved in glucose catabolism. Upon a shift of glucose-grown cells to media containing methanol or oleate the number and size of the peroxisomes rapidly increases, because the synthesis of peroxisomal enzymes involved in the catabolism of these compounds are induced.

Until recently, the generally accepted model of peroxisome formation was the so called "growth and division" mode of peroxisome multiplication. Due to incorporation of newly synthesized peroxisomal membrane and matrix components in pre-existing peroxisomes, these organelles increase in size. When the mature size of the organelle has been reached this organelle divides. This model explains why always at least one peroxisome is present per cell and the need to tightly regulate peroxisome segregation/inheritance during cell division.

However, the growth and division model fails to explain the formation of new peroxisomes in mutant cells that fully lack peroxisomal structures upon transformation of the mutant with the corresponding *PEX* gene. Indeed, recent studies showed the existence of an alternate pathway, by which new peroxisomes arise from the endoplasmic reticulum. Still it is a matter of debate whether this pathway is simply a back-up mechanism or co-exists with a growth and division mode in normal wild type cells.

The aim of this thesis is to get further insight in the process of peroxisome formation, with emphasis on the proteins involved in peroxisome division in yeast.

Chapter 1 summarises the current knowledge of peroxisome division and inheritance in yeast and mammals.

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Chapter 2 describes the function of two dynamin related proteins in controlling peroxisome number in baker's yeast (Saccharomyces cerevisiae). This yeast contains three dynamin related proteins (DRPs), which are proteins known to be involved in membrane fission and fusion processes. These include Vps1p, Dnm1p and Mgm1p.

Earlier studies suggested that only Vps1p is involved in regulating peroxisome abundance in baker's yeast. Indeed, we found that in glucose-grown yeast cells lacking Vps1p, the number of peroxisomes is reduced and the size of the organelles increased. However, our results demonstrate that deletion of the DNM1 gene, known to be important for fission of mitochondria, also results in a strong reduction of peroxisome numbers. This effect was however predominantly evident when cells were grown at peroxisome inducing growth conditions, i.e. in media containing oleate. Furthermore, cells of a strain carrying deletions in both VPS1 and DNM1 (dnm1 vps1 cells) had strictly a single peroxisome, both when cells were grown on glucose or on oleate. Normal peroxisome fission is probably blocked in such cells. Time lapse imaging suggested that in budding yeast cells the single peroxisome ultimately divided, a process that most likely occurred during cytokinesis. Localization studies by fluorescence microscopy, using a Dnm1-GFP fusion protein, showed that this proteins is localised at mitochondria and peroxisomes. These data suggest that in addition to Vps1p, also Dnm1p plays a crucial role in controlling peroxisome numbers.

Chapter 3 deals with the role of S. cerevisiae Mgm1p, another dynamin-like GTPase, in controlling peroxisome numbers. Like dnm1 cells, also mgm1 cells contained reduced numbers of peroxisomes when they were grown in the presence of oleate, but not during growth on glucose. In glucose or oleate-grown cells of a dnm1 mgm1 double deletion strain peroxisome numbers were strongly reduced, resulting in cells containing generally only one peroxisome. The majority of dnm1 mgm1 cells possessed a strongly constricted and tubulated peroxisome. Western blotting experiments, however, revealed that in dnm1 mgm1 cells the levels of Vps1p were strongly reduced, compared to WT controls and dnm1 or

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mgm1 single deletion strains. However, this residual VPS1 amount contributed to the peroxisome phenotype in the *dnm1 mgm1* cells as an reinforced peroxisome phenotype was observed in cells of a *dnm1 mgm1 vps1* triple mutant. In oleate-induced cells of this triple mutant a reinforced peroxisome phenotype was observed in that they showed longer peroxisome extensions that frequently formed constrictions and branches. The most remarkable event of these structures was that they were vigorously moving in the developing bud.

In Chapter 4 studies are described that aimed to analyse the role of Vps1p and Pex11p in peroxisome fission in the methylotrophic yeast *Hansenula polymorpha*. Previous studies showed that, in addition to the DLP's also the peroxisomal membrane bound peroxin Pex11p plays a key role in controlling peroxisome size and abundance in *S. cerevisiae*. Surprisingly, deletion of the *VPS1* gene in *H. polymorpha* did not affect peroxisome size or number, suggesting that Vps1p does not play an important role in regulating peroxisome numbers in *H. polymorpha*. Localization studies however, showed that a minor portion of Vps1p is localized at the peroxisomal membrane, like in *S. cerevisiae*.

Deletion of the *PEX11* gene in *H. polymorpha* resulted in an decrease in peroxisome numbers in conjunction with a strong increase in the size of the organelles, when the cells were grown at peroxisome-inducing growth conditions (i.e. in the presence of methanol). Interestingly, when *H. polymorpha pex11* cells were grown on glucose, a significant fraction of the mother cells failed to retain the peroxisome during budding of the cell. As a consequence, the single peroxisome from the mother cell moved into the newly formed bud. Conversely, during growth of *pex11* cells on methanol, frequently the single, large peroxisome was retained in the mother cell, resulting in the formation of daughter cells that lacked a peroxisome. Upon prolonged cultivation, however, these newly formed cells contained a peroxisome again, which may be derived from the endoplasmic reticulum.

Taken together, our data suggests that Pex11p plays a role in controlling peroxisome number, size and inheritance in *H. polymorpha*.

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Chapter 5 focuses on the isolation and characterization of the *H. polymorpha* *PEX17* gene. Pex17p was shown to be a peroxisomal membrane protein in *S. cerevisiae* and *Pichia pastoris*. Earlier studies suggested that Pex17p, along with two other peroxins, Pex13p and Pex14p, forms a docking complex on the peroxisomal membrane for the receptor proteins of peroxisomal matrix proteins. Using fluorescence microscopy we show that a fusion protein consisting of *H. polymorpha* Pex17p and GFP localizes to peroxisomes. Deletion of the *H. polymorpha PEX17* gene resulted in the formation of a few, small peroxisomes per cell which still contained some matrix proteins. However, the bulk of the peroxisomal matrix proteins were mislocalized to the cytosol in these cells. Our data indicate that unlike *S. cerevisiae* and *P. pastoris*, *pex17* cells of *H. polymorpha* contain peroxisomal structures that are still capable of importing matrix proteins.

Chapter 6 describes detailed morphological and biochemical analyses of all available *H. polymorpha pex* mutants using cells that were grown at highly controlled conditions, namely in chemostat cultures. These studies indicate that *H. polymorpha pex* mutants can be categorized in four distinct morphological classes. The first class consists of *pex* mutants that harbor relatively normal peroxisomes (*pex7, 17, 20*). The second class contains mutants that have small peroxisomal membrane remnants (the so called ghosts) that have a proteinaceous matrix (*pex2, 4, 5, 10, 12, 14*). Cells of class three mutants also have peroxisomal membrane structures, but these are virtually empty (*pex 1, 6, 8, 13*). Finally, class four mutants lack any recognizable peroxisomal membrane structures (*pex3, 19*).

Interestingly, the peroxisome number and volume fraction was significantly higher in the chemostat grown *H. polymorpha pex17* cells relative to *pex17* cells grown in flasks in batch cultures (Chapter 5). This suggests that Pex17p does not play an important role in peroxisomal protein import, but may be important for the efficiency of this process.

Western blot analysis revealed strong variations in Pex5p and Pex20p levels in the mutants under study. In *H. polymorpha pex1, pex4* and *pex6* cells the levels

of these peroxins were very low relative to WT controls. Most likely this is the result of degradation via the ubiquitin/proteasome pathway, which occurs in mutant cells defective in receptor recycling.

Pex5p levels were also reduced in *H. polymorpha pex20* cells. Conversely relatively low amounts of Pex20p were detected in *H. polymorpha pex5* cells. Biochemical studies indicated that both peroxins physically interact, which may result in mutual stabilization.