HANSENULA POLYMORPHA PEX19p IS ESSENTIAL FOR THE FORMATION OF FUNCTIONAL PEROXISOMAL MEMBRANES AND PEROXISOME SEGREGATION

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Peroxisomal membrane proteins are posttranslationally inserted into the organellar membrane. Pex19p is a protein that has been implicated to play a role in sorting of newly synthesized peroxisomal membrane proteins (PMPs). This assumption was based on the findings that Pex19p physically interacts with several PMPs and is predominantly localized to the cytosol, whereas a minor portion is associated with peroxisomes.

We have cloned and characterized PEX19 gene of the yeast Hansenula polymorpha [1]. In a constructed pex19 disruption strain peroxisomal membrane structures were not detectable. PMPs were found in the cytosol (Pex3p), mitochondria (Pex14p) or strongly reduced to undetectable levels (Pex10p). However, by overproduction of the PMP Pex3p or a fragment of this protein fused to green fluorescent protein (Pex3N50.GFP), the phenotype of pex19 cells was suppressed and peroxisomal membrane structures were observed to which PMPs were normally sorted again. These data indicate that HpPex19p is not essential for sorting and insertion of PMPs. Instead, our data are consistent with a function of Pex19p in membrane protein assembly and function.

Because the Pex3N50.GFP fusion protein was correctly sorted to peroxisomal membranes in H. polymorpha pex19, we could easily visualize these organelles by fluorescence microscopy. Upon comparing pex19 cells with identically grown WT cells producing the same fusion protein, a striking difference in distribution of peroxisomes was observed. Also, during budding of the cells, the peroxisomes present in pex19 cells failed to move to the newly developing buds, whereas organelle segregation was very evident in WT control cells. These data indicate that Pex19p is also important for proper organelle positioning and segregation in yeast.

Fluorescence microscopy revealed that buds that fail to inherit peroxisomes form new organelles in an alternative mode. First, Pex3N50.GFP is sorted to the nuclear envelope. At a later stage, GFP fluorescence concentrates at a bright spot and pinches off from the nuclear envelope and develops into a new peroxisome.