Pex5p, a guide for import of proteins into peroxisomes
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Citation for published version (APA):

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Summary

Peroxisomes are subcellular organelles that are enclosed by a single membrane. The peroxisomal lumen harbors the enzymes of a number of important metabolic pathways, one of which is the β-oxidation of fatty acids. The importance of peroxisomes is illustrated by the existence of several severe inheritable diseases in man that are caused by the loss of one or several peroxisomal functions.

Peroxisomes are present in almost every eukaryotic cell. Therefore, an important question deals with the mechanism that ensures that peroxisomes are present in each daughter cell after cell division. Currently, two models exist to explain this. Peroxisomes might either originate from the endoplasmic reticulum or might be maintained by growth and division of pre-existing peroxisomes.

The enzymes that catalyze the reactions inside the peroxisomal lumen are synthesized in the cytosol. To reach their correct destination, these matrix proteins are equipped with a peroxisomal targeting signal (PTS). Two of these targeting signals have been well characterized: type I (PTS1) and type II (PTS2). A PTS consists of a few amino acids, which are recognized by special mobile receptor proteins that are present in the cytosol. The receptor proteins for PTS1 and PTS2 are Pex5p and Pex7p, respectively. Binding of PTS-containing proteins in the cytosol by these receptors is the first step in the import process. In the next step, the receptor-PTS-protein complex docks at the peroxisomal membrane. The PTS-protein is released and translocated across the peroxisomal membrane into the peroxisomal lumen. The receptor protein recycles back to the cytosol where it can perform another round of PTS-protein binding. Since peroxisomal protein import has been conserved during evolution, we made use of Saccharomyces cerevisiae (bakers yeast) as a model organism in the experiments described in this thesis to study the import of matrix proteins into peroxisomes. In this way a better understanding of some human diseases, which are caused by malfunctioning of peroxisomes, can be obtained. The advantage of this single-cell organism is the availability of a large number of genetic tools, which makes it possible to manipulate the protein-import process.

The protein that played a central role in this research was Pex5p, the import receptor for PTS1-containing proteins. There is a direct interaction between the PTS1 and Pex5p. The PTS1 consists of three amino acids at the carboxyl terminus of a peroxisomal matrix protein. In most cases this is S-K-L, but other functional variants are known. To identify the exact binding site for PTS1-containing proteins on Pex5p, a library of Pex5p mutants was screened for those mutants that were affected in the
interaction with the PTS1 (chapter 2). The sites of these mutations were placed in a model for the Pex5p structure, which was created by using another protein that has a structure similar to that of Pex5p. This made it possible to identify the binding site for the PTS1 more precisely. We found that the tetratricopeptide repeat (TPR) domain of Pex5p mediates the binding of the PTS1. The TPR domain of Pex5p consists of six TPR motifs, which form two entities of three TPR motifs, TPR1-3 and TPR5-7. Both are essential for the interaction with the PTS1, but the way in which the PTS1 is bound by these TPR entities differs. Amino acids in Pex5p that are important for the interaction with PTS1 were identified.

The mode of interaction between Pex5p and the PTS1 is conserved between man and *S. cerevisiae*. By comparing the details of this interaction in both species, it was shown that similar residues in Pex5p mediate the interaction with the PTS1 (chapter 3).

Although most peroxisomal matrix proteins possess either a PTS1 or a PTS2, some exceptions are known, for example *S. cerevisiae* acyl-CoA oxidase (Pox1p). The targeting of Pox1p to peroxisomes was studied and this was shown to be dependent on Pex5p, despite the absence of a PTS1 (chapter 4). However, Pox1p uses another binding site on Pex5p than PTS1-containing proteins. Mutations in the PTS1-binding site of Pex5p that affected binding of PTS1-containing proteins and resulted in their mislocalization to the cytosol did not have an effect on the binding and localization of Pox1p. The binding site on Pex5p for Pox1p was identified and was shown to be located outside the TPR domain. These results point to the existence of an additional targeting signal, which would lead to peroxisomal import in a Pex5p-dependent manner.

After binding of peroxisomal matrix proteins in the cytosol, Pex5p docks at the peroxisomal membrane. Pex5p can bind to several peroxisomal membrane proteins: for example to Pex13p, which contains a Src homology 3 (SH3) domain. This cytosolically exposed SH3 domain mediates the binding of Pex5p. SH3 domains are protein modules that interact with P-x-x-P motifs of other proteins, but such a motif is not present in Pex5p. By mutating amino acids in a small area of Pex5p, it was shown that a region that forms an α-helix mediates the binding to Pex13p-SH3 (chapter 5).

Pex14p, another peroxisomal membrane protein, forms the classical P-x-x-P ligand of Pex13p-SH3. The complex formed by Pex13p, Pex14p, and Pex5p was studied in more detail and it was shown that Pex5p and Pex14p bind the SH3 domain of Pex13p at different sites. Pex5p mutants were used in a suppressor screen to identify the Pex5p-binding site on Pex13p-SH3. Combining the positions of the suppressor mutations in Pex13p-SH3 with a structural model of the Pex13p-SH3 domain indicated that Pex5p uses a novel binding site on the SH3 domain. The SH3 domain of
Pex13p is one of the first examples of an SH3 domain that is able to bind two different ligands at the same time.