Receptor-mediated import of proteins into peroxisomes

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Summary

Peroxisomes are organelles that are involved in a number of metabolic processes in the cell. These organelles are present in virtually all eukaryotic cells. In man, defects in peroxisomal function can result in serious disease, in which a large number of organ functions is affected. Several peroxisomal disorders in man are can be caused by (complete or partial) failure in the biogenesis of peroxisomes. The biogenesis of peroxisomes is mediated by a number of proteins named Pex proteins or Peroxins. The two major aspects in peroxisome biogenesis are 1) the formation of the peroxisomal membrane and insertion of proteins into this membrane 2) the transport of proteins that are synthesized in the cytoplasm and that need to find their way across the peroxisomal membrane to reach the peroxisome lumen. Although the amount of newly identified Peroxins that are involved in the import of proteins into peroxisomes is still growing, the actual mechanism of protein import is still unknown.

This thesis focuses on the factors that are involved in the import of peroxisomal proteins in the yeast *Saccharomyces cerevisiae* (baker's yeast). The peroxisomal protein import system is formed by a number of Peroxins. Some of these Peroxins are able to interact with each other and take part in a complex of proteins. The Peroxin Pex5p can bind to a specific targeting signal sequence, the PTS1. A protein that carries a PTS1 is depending on Pex5p for its targeting to the lumen of the peroxisome. In most organisms Pex5p is localized both in the cytoplasm and at the peroxisome. It is assumed that Pex5p acts as a mobile receptor that picks up newly synthesized PTS1-proteins in the cytosol and brings them to the peroxisome. At the peroxisomal membrane the receptor binds two membrane proteins, Pex13p and Pex14p. Here, the PTS1-protein is handed over for transport across the membrane.

Pex13p is a Peroxin that contains an SH3 domain. SH3 domains are generally found in proteins that are involved in highly dynamic processes in the cell. Thus far Pex13p is the only SH3-domain containing protein identified to play a role in the import of proteins into an organelle. SH3 domains bind specific sequences, called PXXP motifs. One of the SH3 binding partners, Pex14p, contains such a PXXP motif. The second binding partner of the Pex13p SH3 domain is Pex5p.

The complex that is formed by Pex13p-SH3, Pex14p and Pex5p is described in more detail in chapters 2 and 3. Specific mutations that disrupt the interactions between Pex13p-SH3 and Pex5p or Pex14p show that 1) the interaction between the two membrane proteins Pex13p and Pex14p is essential for the import of peroxisomal proteins and that 2) Pex14p and Pex5p bind the Pex13p SH3 domain at different sites. Secondary structure analysis combined with the analysis of mutations in Pex5p and Pex13-SH3 predicts a novel interaction between the Pex13p SH3 domain and the Pex5p ligand. This interaction is not essential for the association of Pex5p to the peroxisome.
Chapter 4 deals with several factors that determine the peroxisomal localization of Pex5p. One of these factors is the carbon source on which yeast cells grow. Furthermore, Pex14p appears to be essential for peroxisomal localization of Pex5p. The results suggest a certain sequence of events in the first steps in peroxisomal protein import.

Finally, chapter 5 focuses on a protein that does not contain a recognizable PTS sequence. This protein is nevertheless imported into the peroxisome. The import appears to be dependent on Pex5p, but not on the Pex5p bindingsite for PTS1 sequences. This suggests that Pex5p can also bind another, yet undefined signal sequence and that Pex5p is capable to act as a receptor for proteins with this signal sequence.