6 Abstract

1. Two Hybrid studies with N-and C-terminal deletions of Pex13p showed that the first 55 amino acids of Pex13p are sufficient to bind the PTS2-recognition protein Pex7p. This binding between Pex13p and Pex7p could also be demonstrated through a co-immunoprecipitation. An *in vivo* binding study proved direct binding between the first 100 amino acids of Pex13p and His_6-Pex7p.

2. $Pex13p_{1-100}$ could also interact with Pex18p and Pex21p, though this interaction was shown to depend on the presence of Pex7p by two-hybrid studies.

3. The aminoterminal truncation of $Pex13p_{56-386}$ is unable to complement the growth defect of a *pex13*Δ mutant. As a consequence, this aminoterminal region possesses an essential function for the biogenesis of the peroxisome. Immunofluorescence studies demonstrated further, that this region is particularly necessary for PTS2-dependent protein import. As PTS1-signal recognition [Elgersma, 1996 #192; Erdmann, 1996 #24; Gould, 1996 #239] depends on the SH3-domain of Pex13p, two different regions of Pex13p are obviously involved in PTS-receptor recognition.

4. *β*–Keto-acyl-CoA-thiolase (Fox3p) is able to interact with Pex14p in a wild-type strain, though not in a *pex7*Δ or in a *pex18*Δ*pex21*Δ mutant strain. On the other hand, binding could not be observed between Fox3p and Pex13p, suggesting that Pex14p represents the initial docking protein. Docking of PTS2 cargo proteins is obviously dependent on both Pex18p/Pex21p and Pex7p.

5. The amount of Fox3p coprecipitated with Pex7p in a *pex18*Δ*pex21*Δ and in a *pex14*Δ*pex18*Δ*pex21*Δ mutant strain was significantly smaller than in a *pex14*Δ mutant strain. Therefore it was concluded that the presence of Pex18p and Pex21p is required previously to the docking of Pex7p to the peroxisomal membrane. The two redundant cytosolic proteins Pex18p and Pex21p seem to play an essential role in the formation of an import competent PTS2 substrate complex.

6. The ability of Pex7p for binding to Pex14p was analyzed by *in vitro* binding with bacterially expressed MBP-Pex7p and His_6-Pex14p in the absence of Pex18p/Pex21p. The binding between these proteins thus occurs in a direct manner.

7. Pex7p binds a synthetic PTS2-Protein *in vitro*, implicating that Pex7p alone is sufficient to recognize the PTS2 targeting signal.

8. A second binding site for Pex14p could be identified in Pex13p using the yeast two-hybrid system. This second binding site comprises amino acids 223-258 of Pex13p. In contrast to the SH3-domain, this binding does not depend on the prolin-rich motif of Pex14p.

9. A polyclonal antibody against Pex19p could be generated from heterologously expressed and purified GST-Pex19p. Its specificity could be demonstrated.

10. The binding between Pex19p and Pex13p could be shown in a two-hybrid assay. A truncated version of Pex13p, representing the amino acids 173-258, was shown to be sufficient for Pex19p binding *in vivo*. The incubation of a Pex13p peptide-scan with GST-Pex19p could narrow this binding region for Pex19p down to amino acids 203-213 of Pex13p.

11. Searching for a probably common membrane-protein targeting signal, a Pex13p peptide, comprising the sequence 203-IMKFLKKILYR-213, was analyzed by substitutions. This studies elucidated that the leucine residues in position 207 and 211 seem to be particulary important for the ability for binding by Pex19p.

12. A revised model for the PTS2-dependent import of matrix proteins and its sequential steps was developed (Abb. 4.1).