101 Abstract

6 Abstract

 In the course of this study Pex7p, Pex14p and Pex8p were identified as new interaction partners of Pex8p, by means of two-hybrid-analysis.
Interactions of further peroxines of the docking-machinery, to peroxines of the zincfinger-class and of other functional groups were not identified.

- The region of interaction of Pex8p and Pex5p, Pex8p and Pex14p was narrowed down to amino acids 146-586 of Pex8p.
- It was not possible to narrow down the region of interaction of Pex8p to Pex7p. Pex7p needs a full length Pex8-protein for interaction.
- For interaction with Pex8p (146-586 aa) Pex5p requires the amino acids 1-312. The TRP-domains of Pex5p are not required for the interaction between Pex5p and Pex8p.
- The interaction between Pex8p and Pex14p is influenced by peroxines of the "cargo-receptor-binding-group", the "docking-machinery-group" and the "receptor-translocation-group", and is therefore indirect.
- The interactions between Pex8p and Pex5p, as well as between Pex8p and Pex7p are not mediated by peroxines of the investigated functional groups and are therefore likely to be direct.
- By means of mutationanalysis of Pex8p, three mutations were identified, which prevent interactions between Pex8p and Pex5p, Pex7p and Pex14p in different ways. Mutation Pex8pM-A(S231P) prevents the interaction to Pex5p, Pex7p and Pex14p. Mutation Pex8pM-C(A238V) prevents the interaction to Pex5p and Pex14p. Mutation Pex8pM-D(T327P) prevents the interaction to Pex7p and Pex14p. Therefore, both receptors seem to be necessary for interaction between Pex8p and Pex14p. The Pex8p-mutant can be complemented by all mutated Pex8-proteins. Moreover, fluorescently labelled cargoproteins are imported into peroxisomes in complemented *pex8*Δ-cells. On the basis of the mutationanalysis of Pex8p it is likely, that Pex8p induces the release of the cargo-receptorcomplex due to changes of the receptor conformation.
- In spite of the presence of a PTS-signal in Pex8p, the targeting of Pex8p to the peroxisomal membrane is independent of the PTS-receptors. This is

102 Abstract

further supported by extraction analysis of the protein from peroxisomal membranes.

• In the absence of Pex5p, Pex8p can be degraded by Proteinase K. Therefore Pex5p does not influence the targeting of Pex8p but indirectly influences its approachability to the cytosolic components.