Krupp, A. (2003); "Interaction of the Peroxisomal Membrane Protein Pex13 with Pex5, Pex7 and Pex14", 3rd year project, School of Biomedical and Biomolecular Science, Griffith University

Abstract

Peroxisome biogenesis is a process regulated by a number of genes, termed PEX genes, which encode proteins known as peroxins. The peroxins are responsible for the formation, proliferation and import of matrix proteins which in turn are essential for the metabolic processes within the peroxisome. The project presented in this report deals with particular protein interactions prior to the translocation of matrix proteins. This step is called docking and involves the peroxins Pex13, Pex14, Pex5, Pex7 and possibly other peroxins in mammals. Recently, Otera and co-workers (2002) found a discrepancy in the accepted model of the docking event as it has been reported in yeast. It has been known for some time that Pex14 is the initial docking site for PTS1- and PTS2-protein receptor complexes at the peroxisomal membrane where these complexes are subsequently translocated across the peroxisomal membrane in an unknown manner and the receptors recycled back into the cytoplasm in a process thought to involve Pex13. In yeast, the PTS1- and PTS2-pathways has been shown to be diverged and involve the binding of different regions of Pex13. Pex5 has been shown to bind the SH3 domain of Pex13 independently (Urquhart et al. 2000) as has Pex14. Pex7 has been shown to interact with the N-terminal region of Pex13 (Girzalsky et al., 1999; Stein et al., 2002). Pex5 has never been shown to bind the SH3 region of Pex13 in mammals however, a recent study by Otera et al. (2002) has shown that Pex5 binds at the N-terminus of Pex13. Recent studies in our laboratory, using a mouse model system based on the deletion of Pex13 in conjunction with peroxisome rescue analysis suggest that PTS1 import does occur, albeit at reduced levels. The study presented here aims to establish if the interactions seen in Otera and co-workers (2002) *in vitro* binding assay could be emulated or to explain the discrepancy seen *in vivo* in peroxisome rescue experiments in fibroblasts derived from $Pex13^{-/-}$. To achieve this, three different plasmids containing either the N-terminal, C-terminal or the full-length coding sequence of Pex13 were constructed to express these Pex13 fragments as GST-fusion proteins and as a backup the same sequences were also cloned into vectors expressing either C-terminal or N-terminal hexa-His tags.

The Pex13-GST fusion proteins were expressed, purified and used to perform pull-down assays by immobilising the Pex13-GST fusion proteins and incubating them with its reported interaction partners Pex5, Pex7 and Pex14. Binding was analysed by SDS-PAGE and western blotting.

The full-length Pex13 and the amino-terminal fragment of Pex13 showed binding to Pex14. Pex7 was shown to weakly bind all Pex13-GST fusion proteins solely or in the presence of Pex5 and Pex14. If Pex7 was incubated together with Pex5 added to the assay, no binding was observed.