Export-driven Protein Import into Peroxisomes

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The peroxisomal matrix protein import is facilitated by cycling receptor molecules that shuttle between the cytosol and the peroxisomal membrane. In the yeast *Saccharomyces cerevisiae* the import of proteins harbouring a peroxisomal targeting signal of type II (PTS2) is mediated by the receptor Pex7p and its co-receptor Pex18p. Under oleic-acid condition, Pex18p turned out to be responsible for the import of the PTS2-carrying thiolase (Fox3p). Here we demonstrate that Pex18p undergoes two different kinds of ubiquitin modifications. Ubiquitination that forces rapid Pex18p-turnover by proteasomal degradation takes place at conserved lysine residues. In contrast thereof, a second type of ubiquitination depends strongly on a conserved cysteine residue near the extreme Pex18p-N-terminus. To elucidate the topology of the PTS2-import complex, we performed protease-protection assays. Under wild-type conditions, Pex18p is accessible to exogenously added proteases, whereas Pex7p remains stable under these conditions, most likely because it is protected by the peroxisomal membrane. In mutants where the receptor export is blocked, the topology of both, Pex7p and Pex18p changes. Pex7p becomes protease accessible whereas Pex18p is protected. This finding indicates that Pex18p export drives the import of the cargo bound PTS2-receptor, which supports the idea of an export-driven import model.