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ADDENDUM 1 TO CHAPTER 2

HAS *S. CEREVISIAE* PEX15P SEEN THE ENDOPLASMIC RETICULUM EN ROUTE TO THE PEROXISOME?

INTRODUCTION

Pex15p is one of the proteins that has been suggested to travel via the ER to peroxisomes, a suggestion which is based on the observation that overexpression of this protein leads to profound ER membrane proliferation (karmellae, Elgersma *et al.*, 1997). However we indicated in chapter 2 that karmellae formation in *S. cerevisiae* is not an ER protein specific event and that it can even be induced by expression of membrane proteins that never pass the ER. This finding suggests that the formation of karmellae upon Pex15p overexpression is not a valid indication for the passage of Pex15p through the ER. It fails therefore as an argument for the involvement of the ER in peroxisome biogenesis. Here we describe another experiment of which the outcome is in line with this conclusion.

This experiment is based on the knowledge that in *pex3* or *pex19* mutants, integral peroxisomal membrane proteins are rapidly degraded after their synthesis (Hettema *et al.*, 2000). Since Pex15p is an integral peroxisomal membrane protein, it is not surprising that it is degraded in *pex3Δ* cells. At least, this is the case when it is expressed at the endogenous level. When Pex15p is expressed at a level at which karmellae are formed surprisingly this no longer holds true. This indicates that the induction of karmellae formation by overexpressed Pex15p is not a reflection of what happens with Pex15p when it is expressed at a physiological level.

MATERIALS AND METHODS

See also materials and methods of chapter 2. *pex3Δ* and *pex6Δ* cells (same as BJ1991 except *pex3/6:Leu2*) were made by homologous recombination. The cells were precultured on selective 0.3% glucose medium for 24 hours followed by 16 hours on selective oleate medium before they were analysed by EM or Western blotting (see chapter 4).

RESULTS

At the endogenous expression level Pex15p, like other integral PMPs, depends on the presence of Pex3p and Pex19p for localisation to the peroxisomal membrane, since in *pex3Δ* or *pex19Δ* cells Pex15p is mislocalised to the cytosol and rapidly degraded (Hettema *et al.*, 2000). When expressed under control of the catalase promoter from a multi copy plasmid, Pex15p is overproduced and ends up in karmellae. To understand what happens with overexpressed Pex15p in the absence of Pex3p we expressed NH-tagged Pex15p under control of the catalase promoter in the *pex3Δ* strain and induced the cells on oleate medium. As determined by immuno EM using antibodies against the NH-tag, NH-Pex15p was found to be present in karmellae as well as in the nuclear envelope (Figure 1A). As a control NH-Pex15p was also expressed in another *pex* mutant, *pex6Δ*. This too resulted in accumulation of NH-Pex15p in karmellae (Figure 1B). Obviously overexpressed NH-Pex15p is not rapidly degraded in *pex3Δ* cells. This is confirmed by the Western blot shown in Figure 2.

DISCUSSION

The presence of the NH-tag in these experiments is not likely to have influenced the results, since NH-Pex15p can rescue the growth defect of *pex15Δ* on oleate containing medium and in wild type cells Pex15p and NH-Pex15p overexpression both lead to karmellae formation.

Overexpression of Pex15p in wild type, *pex3Δ* and *pex6Δ* cells all resulted in the formation of karmellae. This identical phenotype indicates that the absence of Pex3p or Pex6p does not influence the karmellae inducing capacity of Pex15p. Moreover overexpressed Pex15p is definitely not degraded to a large extent in *pex3Δ* cells. This result is in contradiction with the expectation that in *pex3Δ* cells integral membrane

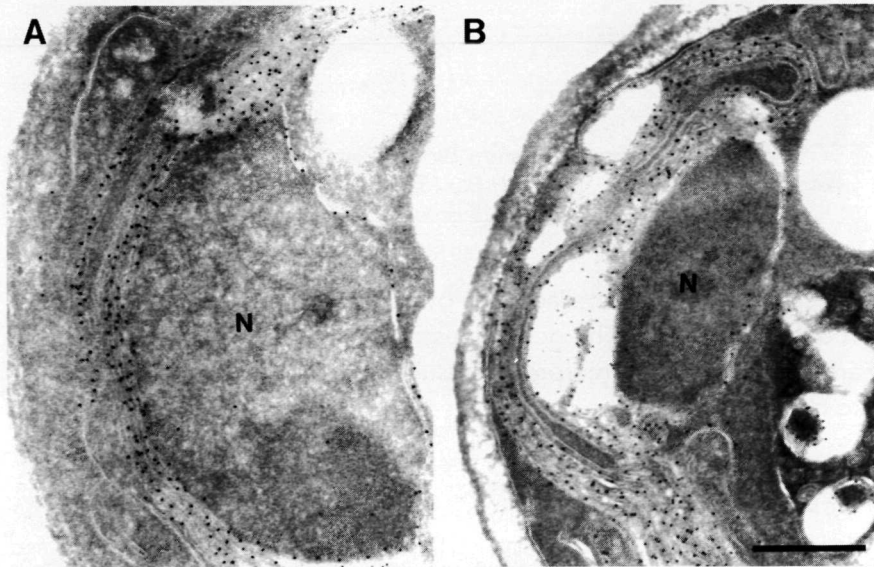


Figure 1: Electron microscopic analysis of A) *pex3Δ*, and B) *pex6Δ* cells after overexpression of NH-Pex15p (behind the catalase promoter, 2μ plasmid). In both mutants NH-Pex15p expression leads to karmellae formation. α -NH is used, showing the presence of NH-Pex15p in karmellae. The bar is $0.5\ \mu\text{m}$.

proteins are degraded, a phenomenon which does occur when Pex15p is expressed at a physiological level (controlled by its own promoter). In this latter case the remaining Pex15p is found to be present in the cytosol whereas the overexpressed Pex15p resides in karmellae (Hetteema *et al.*, 2000). Obviously, overexpressed Pex15p shows different behaviour than endogenously expressed Pex15p. This result indicates that the localisation of Pex15p in extended ER membranes is caused by overexpression of the protein and probably does not represent the accumulation in the ER of a naturally occurring sorting intermediate. Combined with the results in chapter 2 this means that the hypothesis that Pex15p travels to the peroxisome via the ER can be seriously questioned. Of course this does not rule out the possibility that the ER is involved in peroxisome biogenesis or even that Pex15p or other peroxisomal membrane proteins might travel via the ER to peroxisomes.

It is interesting to note that the absence of Pex6p does not influence the induction of karmellae formation (interesting since we know now that Pex15p and Pex6p interact, see chapter 4).

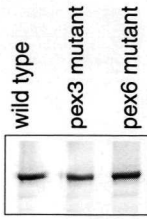


Figure 2: Western blot showing equal amounts and stability of NH-Pex15p in wildtype, *pex3Δ* and *pex6Δ* cells. α -pex15 is used.

The formation of karmellae upon overexpression of Pex15p is therefore probably an artefact. The general lesson that can be learned here is that care must be taken in interpreting results of experiments in which proteins are not expressed at their natural levels.

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