INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Programme of Study	:	Ph.D.
Thesis Title	:	Molecular characterization of the organelle contact facilitating protein Pex30 and its role in peroxisome dynamics in *Saccharomyces cerevisiae*
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Thesis Submitted to the Department/ Center	:	Biosciences and Bioengineering
Date of completion of Thesis Viva-Voce Exam	:	27-01-2023
Keywords for description of Thesis Work	:	Peroxisomes, Phosphorylation, ER, Pex30, Mass spectrometry, Domain, Dysferlin, Yeast

SHORT ABSTRACT

Peroxisomes are single membrane-bound organelles found in most eukaryotes. They perform diverse cellular functions depending on the metabolic requirements of the cell. Scavenging harmful reactive oxygen species and β-oxidation of fatty acids are the most well-characterized functions of peroxisomes. They are dynamic in nature whose number and function may vary according to the need of the cell. Peroxisomes interact with other surrounding organelles like mitochondria, lipid droplets, endoplasmic reticulum, *etc* to optimize their multiple cellular functions. Several peroxisomal proteins have been identified with a role in various facets of peroxisome biogenesis and functions. Pex30 is one such peroxisomal protein that resides in the endoplasmic reticulum and associates with peroxisomes to regulate their biogenesis. It is a 58 kDa protein that consists of an N-terminal reticulon homology domain and an uncharacterized C-terminal dysferlin domain. Recent studies have elucidated the role of Pex30 in the formation of pre-peroxisomal vesicles and lipid droplets from the endoplasmic reticulum. Interestingly, it also associates with the ER reticulon proteins and functions in the maintenance of endoplasmic reticulum morphology.

In this study, we characterized the mechanism involved in the dual distribution of Pex30 and its function in peroxisome biogenesis. For this, we looked at the role of uncharacterized interacting proteins of Pex30, the role of post-translational modifications and the role of various domains of the protein in its distribution and functions. Pex30-GFP displayed both punctate and reticulate distribution of the protein as reported in previous studies whereas GFP-Pex30 cells exhibited only the reticulate phenotype suggesting that N-terminal tagging with GFP disrupted the punctate distribution of Pex30. Further our microscopic analysis revealed an interesting difference in the distribution of Pex30-GFP in cells grown in glucose-containing media and peroxisome-inducing media. Reticulate distribution of GFP around the cell periphery along with discrete GFP puncta was observed in almost all the cells cultured in glucose-containing media. On the other hand, cells grown in peroxisome-inducing media exhibited two distinct phenotypes viz. only reticulate and both reticulate and punctate. In line with this, western blot analysis revealed reduced expression of Pex30 in peroxisome-inducing media as compared to glucose-containing media. We further analyzed the distribution and expression of the protein in strains deleted for Pex30 interacting proteins such as

Pet10, Erg6 and Mdm10. Our data indicate that the distribution and expression levels of Pex30 remain unaltered in the deletion strains. Quantification data indicate that cells lacking Mdm10 exhibit significant increase in peroxisome number as compared to wild-type cells.

Further, to understand the role of post-translational modifications in targeting and function of the protein, Pex30 was purified and the secondary structure was determined by far-UV circular dichroism spectroscopy. Mass spectrometry analysis of the purified protein identified three residues at Threonine 60, Serine 61 and Serine 511 that are phosphorylated. The significance of phosphorylation of Pex30 and its contribution to protein localization was then studied by modifying the phosphorylated residues to non-phosphorylatable and phosphomimetic mutant variants. Microscopy analysis of the mutant variants indicated that alteration in the phosphorylation status did not change the localization of the protein to peroxisomes and endoplasmic reticulum. Notably, a decrease in peroxisome number was exhibited in cells expressing the phosphomimetic variants in peroxisome-inducing media. This indicates that phosphorylation of Pex30 may be crucial in the regulation of peroxisome abundance but not important for its targeting to peroxisomes and endoplasmic reticulum.

To understand whether the domains of Pex30 influence the sub-cellular localization of the protein and the regulation of peroxisome number, we constructed six different truncated versions of the protein. The growth kinetics of cells expressing the truncated variants did not differ significantly in comparison to cells expressing the full-length protein. Interestingly, each of these truncations showed distinct distribution patterns such as punctate, reticulate and cytosolic fluorescence. In addition, variable expression and localization of the truncated variants in both peroxisome-inducing and non-inducing growth conditions were observed. The absence of the entire dysferlin domain or only the C-Dysf leads to an increase in peroxisome number similar to that exhibited by cells lacking the full-length protein. However, our data indicate that the dysferlin domain does not contribute to the reticulate distribution of Pex30.

In conclusion, our study sheds light on some interesting aspects of the regulation of Pex30-mediated peroxisome biogenesis.