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**Research visit at the Department of Plant and Environmental Sciences
University of Gothenburg, Gothenburg, Sweden
October 2008 - May 2009**

Topic: Investigation of organellar contact sites in plants and yeast

Supervisor abroad: A. S. Sandelius

Aim of the visit

Phospholipids are required for membrane formation, organelle assembly and cell organization. Despite their requirement in all cellular membranes, only some organelles, e.g. the endoplasmic reticulum (ER), the mitochondrion and the Golgi, are capable of synthesizing phospholipids. In contrast, peroxisomes lack the capacity of lipid synthesis and therefore depend on lipid supply from other organelles. As peroxisomes are not part of the secretory pathway, mechanism(s) of lipid supply are most likely different from vesicle flux. My ongoing work addressing possible lipid traffic routes to yeast peroxisomes suggests direct membrane contact with other organelles, especially the endoplasmic reticulum and mitochondria [1], as a possible mechanism. My host laboratory developed a method to visualize membrane attachment sites using fluorescence microscopy in plant cells [2]. Applying a special optical tweezer technique the group was able to visualize and measure forces between direct membrane contacts. During my stay I tried to adapt this technique to yeast cells with special emphasis on peroxisomes. Moreover, I got the opportunity to study plant peroxisomes in more detail.

I was able to isolate yeast peroxisomes from a strain expressing a modified GFP-SKL protein that localized GFP exclusively to peroxisomes. Unfortunately, analyses in the fluorescence microscope revealed that the optical tweezers technique was not suitable for yeast peroxisome. Although I could increase size and abundance of peroxisomes in *Saccharomyces cerevisiae* by supplementing the media with oleate, the diameter was still too small for attaching the organelle under the microscope.

In a next step I cultivated *Arabidopsis thaliana* also containing a GFP-SKL construct and established a protocol for plant peroxisome purification according to the yeast standard protocol using a density gradient centrifugation. After successful isolation of plant

peroxisomes I investigated possible membrane contact with other organelles. I was not able to find peroxisomes with ER or mitochondria attached but only with chloroplasts (see Fig. 1). Due to limitations of time I was not able to perform force measurement studies on the isolated organelles.

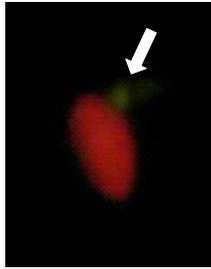
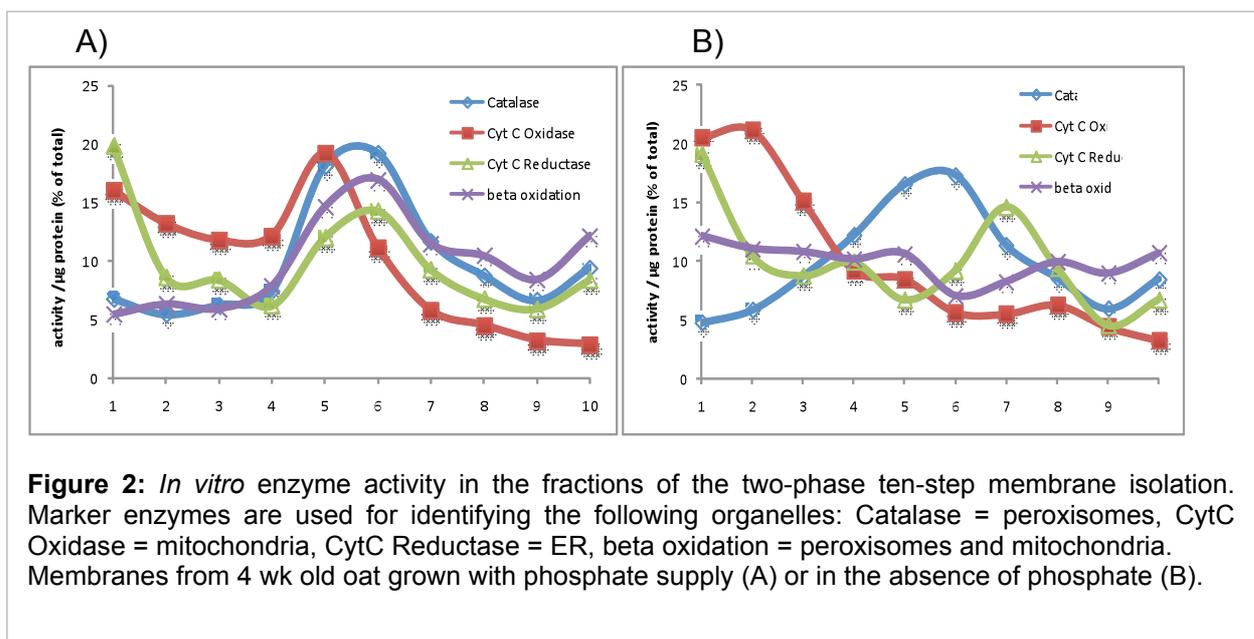


Figure 1: Fluorescence microscopy of a plant peroxisome (green, white arrow) attached to a chloroplast (red). The direct attachment of both organelles was confirmed by using optical tweezers to drag the chloroplast on the slide, the associated peroxisome followed every movement of the chloroplast.

In another experiment I was able to gain more insight into plant cultivation, preparation and cell biology. In an attempt to isolate peroxisomes from oat I was able to learn a new technique of membrane organelle preparation using a two phase system of two aqueous polymers in a ten step isolation [3]. The purity of the membranes was then validated by *in vitro* enzyme assays. This experiment was part of a project investigating the impact of phosphate deprivation on oat lipid metabolism. It was observed previously that in the plasma membrane and tonoplast of oat roots the level of the major phospholipids (PC, PE, PG, PA and PI) was highly reduced while the galactolipid Digalactosyldiacylglycerol (DGDG) was enriched about

7 times when the plants were grown in the absence of phosphate [3]. ER and mitochondria did not seem to contain elevated DGDG levels. In this side project I was supposed to isolate membranes from 4 weeks old oat with and without phosphate supply and investigate the localization of peroxisomes by using an *in vitro* catalase assay as well as other marker enzyme assays for localizing ER and mitochondria (see Fig. 2).



Interestingly I found that organelles from oat grown with phosphate supply seem to have similar membrane polarities as they were not separated by the two phase system (Fig. 2A). All membranes were found in fractions 5 and 6. In contrast, organelles from oat grown in the absence of phosphate clearly localized to different fractions indicating that they must have different membrane polarities (Fig. 2B). Mitochondria were found mostly in fractions 1 and 2 whereas ER seemed to localize mainly in fraction 7. Peroxisomes were found again in fractions 5 and 6 and did not shift. I found that mitochondria and ER from oat grown with and without phosphate showed different behavior in membrane polarity whereas peroxisomes seem to have no difference in membrane polarity. I conclude that the membrane lipid or protein composition must be distinct in peroxisomes. Further lipid and protein analyses must clarify these underlying differences.

References

1. Rosenberger S, Connerth M, Zellnig G and Daum G (2009) Phosphatidylethanolamine synthesized by three different pathways is supplied to the peroxisomes of the yeast *Saccharomyces cerevisiae*. *BBA*, **1791(5)**, 379-387.
2. Andersson MX, Goksör M and Sandelius AS (2007) Optical manipulation reveals strong attracting forces at membrane contact sites between endoplasmic reticulum and chloroplasts, *J. Biol. Chem.*, **282**, 1170-1174.
3. Andersson MX, Larsson KE, Tjellström H, Liljenberg C, Sandelius AS (2005) Phosphate-limited oat. The plasma membrane and the tonoplast as major targets for phospholipid-to-glycolipid replacement and stimulation of phospholipases in the plasma membrane. *J. Biol. Chem.*, **280(30)**, 27578-27586.

