visualize the dynamics of COPII vesicle formation in real time [4]. Bet1p-Cy3—a fluorescently labeled cargo protein—was reconstituted in artificial planar lipid membrane (APLM). When COPII components (Sar1p, Sec23/24p, and Sec13/31p) were sequentially added to the APLM, bright spots from Bet1p-Cy3 were bound with each other, forming large clusters. This indicates that Sec13/31p clustered the complexes that contain the cargo protein. We compared the fluorescent intensity of the clusters that contain the cargo protein in the presence of GTP and a nonhydrolyzable analog (GMP-PNP). When GMP-PNP was used, the fluorescent intensity of the clusters was significantly lesser than that of the clusters using GTP; this indicated the involvement of GTP hydrolysis in the concentration of the cargo in the clusters. Next, when fluorescently labeled non-cargo protein (Ufe1p-ATTO) was reconstituted on the APLM together with Bet1p-Cy3 in a cluster formation experiment, the clusters which were darker than the surroundings were formed on Ufe1p-ATTO. This indicates that Ufe1p-ATTO was excluded from the clusters. On the basis of these results, we discuss about the cargo protein dynamics.

References

[1] Bonifacino J.S. *et al.* (2004) *Cell* **116:** 153–1662.
[2] Matsuoka K. *et al.* (1998) *Cell* **93:** 263–2753.
[3] Sato K. *et al.* (2005) *Nat. Struct. Mol. Biol.* **12:** 167–1744.
[4] Tabata K.V. *et al.* (2009) *EMBO J.* **28:** 3279–3289.

doi:10.1016/j.bbabio.2010.04.153

3P.11 Peroxisomal transporters associated with β-oxidation

Frederica L. Theodoulou[1], Yvonne Nyathi[2], Carlo van Roermund[3], Carine De Marcos Lousa[2], Nicole Linka[4], Xuebin Zhang[1], Richard Haslam[1], Johnathan A. Napier[1], Stephen A. Baldwin[5], Andreas Weber[4], Alison Baker[2]
[1]*Rothamsted Research, Biological Chemistry Department, UK*
[2]*University of Leeds, Centre for Plant Sciences, UK*
[3]*University of Amsterdam Academic Medical Centre, Departments of Pediatrics and Clinical Chemistry, The Netherlands*
[4]*Heinrich-Heine Universität Düsseldorf, Institut für Biochemie der Pflanzen, Germany*
[5]*University of Leeds, Institute of Membrane and Systems Biology, UK*
E-mail: freddie.theodoulou@bbsrc.ac.uk

Peroxisomes perform a range of different functions, including β-oxidation of fatty acids and synthesis and degradation of bioactive lipid-derived molecules. A key feature of peroxisomes is their role in metabolic pathways which are shared between several subcellular compartments, including mitochondria, chloroplasts and cytosol. Transport across the peroxisomal membrane is therefore essential for the coordination of metabolism. Although transport proteins are very likely required for import of substrates and cofactors, export of intermediates and products and the operation of redox shuttles, relatively few peroxisomal transporters have been identified to date. We have identified and characterised two peroxisomal transport systems which are required for β-oxidation in the model plant, *Arabidopsis thaliana*. Peroxisomal Nucleotide Carrier 1 and 2 were identified by homology with the yeast peroxisomal adenine nucleotide carrier and were shown by complementation and *in vitro* uptake assays to catalyse the counter exchange of ATP with AMP [1]. Inducible RNAi lines demonstrated that import of ATP into peroxisomes is essential for activation of fatty acids during seedling establishment and plays a role in other β-oxidation reactions such as auxin metabolism. *Arabidopsis* also contains a single peroxisomal ABC transporter, COMATOSE (CTS), which has been identified in at least four independent forward genetic screens. Analysis of *cts* null mutants has demonstrated that CTS plays key roles in a number of developmental and physiological processes, including germination, seedling establishment, fertility and root growth [2]. We demonstrate that the different roles of CTS *in planta* are separable by mutagenesis [3] and can be related to different biochemical roles, specifically the ability to metabolise distinct substrates such as fatty acids and hormone precursors via β-oxidation. Taken together, these findings strongly suggest that CTS is a broad specificity transporter which mediates uptake of substrates for β-oxidation into the peroxisome. Here, we present biochemical characterisation of heterologously-expressed CTS, provide evidence for its role as a transporter of fatty acyl-CoAs and compare its activity to that of yeast and mammalian homologues.

References

[1] Linka N., *et al.* (2008) *Plant Cell.* **20:** 3241–13257.
[2] Baker A. *et al.* (2009) *Trends Pharmacol. Sci.* **11:** 1360–1385.
[3] Dietrich D. *et al.* (2009) *Mol. Biol. Cell* **20:** 530–543.

doi:10.1016/j.bbabio.2010.04.154