MEETING REPORT

Peroxisomes : novel findings and future directions

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Introduction

The 8th Open European Peroxisome Meeting (OEPM) took place in Aveiro, Portugal, from 22nd to 24th of September 2022. The OEPM is a biannual meeting organized by European researchers working on peroxisome biology. Previous meetings were held in Leuven, Belgium (2006); Lunteren, The Netherlands (2010); Dijon, France (2012); Neuss, Germany (2014); Vienna, Austria (2016); Groningen, The Netherlands (2018); and Bochum, Germany (2020), the latter one as a web conference due to the coronavirus pandemic. The 8th OEPM was attended by 118 researchers (Fig. 1) from 15 countries (Austria, Belgium, Canada, Finland, France, Germany, Israel, Italy, Japan, The Netherlands, Portugal, Spain, Switzerland, UK, and USA).

At every edition of the OEPM, young researchers (graduate students and junior postdoctoral researchers) are selected to present their work to an international audience. The 8th OEPM included 38 exceptional oral presentations distributed over 7 sessions, and 43 outstanding posters.

During the meeting, the "OEPM2022 Peroxisome Research Young Investigator Award", attributed to an

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Fig. 1 Group photo of all the participants of the 8th Open European Peroxisome Meeting (OEPM) in Aveiro, Portugal

early career researcher who was first author of the best publication in the peroxisome field over the last 2 years, was awarded to Susan Kors from the group of Michael Schrader (University of Exeter, UK). The awarded publication provides new insights into the peroxisome–endoplasmic reticulum (ER) association, revealing that the interaction mediated by the peroxisomal membrane protein ACBD5 (Acyl-CoA Binding Domain Protein 4/5) and the ER protein VAPB is regulated by phosphorylation of the FFAT motif and glycogen synthase kinase-3 β (GSK3β) (Kors et al. 2022). Suzan Kors presented the awarded work in the Young Investigator Award lecture. The jury committee for this specific award was constituted by Triana Amen (Swiss Federal Institute of Technology, Switzerland), Einat Zalckvar (Weizmann Institute of Science, Israel), Francesca Di Cara (Dalhousie University, Canada), Johannes Freitag (Philipps-University Marburg, Germany), Marc Fransen (Katholieke Universiteit Leuven, Belgium), Margret Bulow (Life and Medical Sciences Institute, Germany), Myriam Baes (Katholieke Universiteit Leuven, Belgium), Nicole Linka (Heinrich Heine University, Germany), Paul Walton (University of Western Ontario, Canada), Ralf Erdman (Ruhr University,

Germany), Ronald Wanders (Amsterdam University Medical Center, The Netherlands), Stephane Savary (Université de Bourgogne, France), and Sven Thoms (University of Bielefeld, Medical School EWL, Germany).

Peroxisomes are intracellular organelles formed by a single lipid bilayer membrane that surrounds a dense proteinaceous matrix, whose shape and size rapidly changes in response to environmental stimulus (Wanders et al. 2022). As crucial organelles, peroxisomes assume several important functions, including β- and α-oxidation of fatty acids, decomposition of hydrogen peroxide, and synthesis of ether-phospholipids and docosahexaenoic acids (Wanders et al. 2022). To accomplish these functions, peroxisomes interact with several other cellular organelles, such as mitochondria, ER, or lipid droplets (Sargsyan and Thoms 2020; Schrader et al. 2020). In the last decades, peroxisomes have also been shown to play important roles in the contexts of infection and the immune response (Ferreira et al. 2022b; Di Cara et al. 2023), and their dysfunction has been associated with different metabolic disorders, such as the Zellweger syndrome (Wanders 2014), as well as nonmetabolic neurodegenerative diseases (Dorninger et al. 2017), cancer (Kim 2020) and aging (Cipolla and Lodhi 2017).

These and other topics of peroxisome biology were discussed in seven remarkable sessions in this latest edition of the OEPM. A summary of these sessions is presented below.

Session 1: Peroxisome protein sorting

Chairs: Ana Pedrosa, Universidade do Porto, Portugal and **Katharina Reglinski**, **Friedrich-Schiller University Jena, Jena, Germany**

Proteins belonging to the peroxisomal membrane or matrix have to be sorted/targeted to the organelle after their synthesis. Although many of the proteins involved in these targeting pathways are known, the mechanisms through which they accomplish their functions are still not completely understood. The first session of this meeting focused on this topic, with seven talks that provided new and exciting data. Six of these talks are summarized below.

Peroxisome biogenesis has been a matter of debate in the field for many years. The current knowledge supports the existence of two pathways for the organelle's biogenesis: peroxisomes are formed either by growth and division of preexisting organelles with insertion of new peroxisomal membrane proteins (PMPs) as well as matrix proteins, or de novo by budding from ER sites containing some of the PMPs (reviewed in Hettema et al. 2014; Hua and Kim 2016). **Tamara Somborac** (Svetlana Konovalova's laboratory, University of Helsinki, Finland) shed light on this issue with a recent study that focused on the two possible pathways that peroxisomal tail-anchored (TA) proteins may take en route to peroxisomes—via direct delivery or through the ER—thereby posing a question on whether the ER plays a role in this trafficking process. Using biochemical- and microscopy-based approaches, it was shown that the ER is not involved in trafficking of TA proteins to peroxisomes. The results indicate that, in the absence of peroxisomes, TA proteins mainly localize to mitochondria. Thus, the gathered evidence does not support the idea that TA proteins are transported to peroxisomes via the ER in mammalian cells, but rather that mitochondria could be involved in this process.

Most of the session was dedicated to the import of matrix proteins into peroxisomes. The data presented ranged from the characterization of protein–protein interactions between peroxins involved in this pathway and the identification of a new peroxin involved in protein import, to structural studies of the PEX1/PEX6 AAA ATPase complex. The PEX1/PEX6 type II AAA-ATPase complex is the driving force for peroxisomal receptor recycling (Erdmann et al. 1991). Impaired PEX1/PEX6 function results in severe peroxisomal biogenesis disorders (Waterham and Ebberink 2012). **Maximillian Rüttermann** (Christos Gatsogiannis's laboratory, University of Münster, Germany) presented the first

high-resolution cryoEM structures of the PEX1/PEX6 complex from *Saccharomyces cerevisiae* in two different conformations bound to an endogenous substrate. He showed that translocation of the substrate occurs along the central channel formed by rings D1 and D2, but ATP hydrolysis is restricted in the ring D2. The pore II loops of the PEX1/PEX6(D2) subdomains bind the substrate in a canonical staircase arrangement (Puchades et al. 2020). However, PEX1 and PEX6 function in pairs, and the ATPase cycle involves an intriguing uncoupling of a "twin seam" PEX1/PEX6(D2) heterodimer from the staircase. The respective mechanical forces are transmitted to the D1 ring via different interfaces, resulting in alternate widening and constriction of its pore. The data presented highlighted the complex interplay between PEX1 and PEX6 and reveal fundamental differences from homo-oligomeric type II AAA ATPases. We continued to learn more about the peroxisomal AAA ATPases with **Lavanya Mahadevan** (Ralf Erdmann's laboratory, Ruhr-University of Bochum, Germany) who reported the newly identified homologs of AAA ATPases PEX1 and ATAD1 in trypanosome parasites, and discussed how the peroxisomal protein sorting pathway could become a target for therapy of human diseases caused by trypanosomatids. *Trypanosoma* are kinetoplastid parasites that cause the deadly human African trypanosomiasis, Chagas disease, and leishmaniasis, affecting the lives of millions of the global population. These parasites possess unique peroxisome-like organelles called glycosomes, which are essential for the survival of the parasites. Lavanya Mahadevan established conditional knockout (KO) models and showed that ATAD1 is not essential for the parasite's bloodstream form survival, while PEX1 was verified as an essential gene for the survival of the parasite using RNA interference (RNAi) analysis. PEX1 was shown to localize to glycosomes in vivo and the knockdown of PEX1 by RNAi partially resulted in the mislocalization of glycosomal enzymes. Knockdown of PEX1 caused a proteasome-dependent degradation of the cargo receptors PEX5 and PEX7, indicating a well-conserved role of PEX1 in quality control and glycosomal biogenesis. Considering the essentiality of both PEX1 and PEX6 genes (Krazy and Michels 2006), Lavanya proposed the PEX1/PEX6 interaction as a potential drug target against trypanosomiasis. She is currently working toward establishing an in vitro screening to test small molecule inhibitors.

Two talks from this session were centred on PEX5, the shuttling receptor that transports matrix proteins to peroxisomes, and on its interaction with PEX13 and PEX14, two components of the peroxisomal membrane module that accomplish protein import. Peroxisomal matrix proteins can be imported from the cytosol in a folded state, but how they cross the membrane is, to date, poorly understood. **Michael L. Skowrya** (Tom A. Rapoport's laboratory, Harvard Medical School, USA) presented data suggesting

that the peroxisomal import is similar to nuclear transport. Michael and his colleagues found that a selective phase, or meshwork, is formed in the peroxisomal membrane by the conserved tyrosine/glycine-rich YG domain of PEX13. This meshwork resembles that formed by nucleoporin FG repeats in nuclear pores, and is suspended in the membrane by multiple PEX13 molecules of opposite transmembrane orientations. Furthermore, purified YG domains were shown to form hydrogels into which the peroxisomal import receptor PEX5 selectively partitions, using conserved aromatic WxxxF/Y motifs and bringing the cargo along. Michael L. Skowrya concluded the presentation by proposing that PEX5 ferries matrix proteins into peroxisomes through a nuclear pore-like conduit formed by PEX13. **Stefan Gaussmann** (Michael Sattler's laboratory, Helmholtz Zentrum München, Germany) discussed novel binding interfaces among PEX5, PEX13, and PEX14 that were identified and characterized using nuclear magnetic resonance (NMR) spectroscopy and biochemical methods. This work revealed a novel interaction site between the C-terminal cargo-binding TPR domain of PEX5 and the C-terminal intrinsically disordered region of PEX14. Importantly, mutational analysis of this newly identified interface impairs peroxisomal protein import, indicating functional significance of the interaction. Also, a first structural analysis of human PEX13 was reported, which revealed an intramolecular interaction of the PEX13 SH3 domain with a proximal FxxxF motif. This motif was found to modulate the interaction of PEX13 with PEX14 and PEX5. Finally, mutation or deletion of the PEX13 FxxxF motif shows reduced import efficiency, hinting to a fine tuning mechanism in matrix protein import.

A particular highlight of this session was the report of a novel peroxin specifically involved in the peroxisomal targeting signal (PTS) type 2-containing protein import pathway. **Daniel Wendscheck** (Bettina Warscheid's laboratory, University of Würzburg, Germany) presented results of a collaborative project involving the laboratories of Bettina Warscheid (University of Würzburg), Jorge Azevedo (Universidade do Porto), Maya Schuldiner and Einat Zalckvar (Weizmann Institute of Science), Marc Fransen (Katholieke Universiteit Leuven), and Hans Waterham (Amsterdam University Medical Center). The aim of this project is to characterize a phylogenetically conserved protein with a, so far, unknown function. The yeast ortholog was identified as an interactor of PEX18 and subcellular localization studies of a NeonGreen-tagged protein showed a partial peroxisomal localization. Importantly, a deletion mutant displayed a growth defect under peroxisome-proliferating conditions and a cytosolic mislocalization of PTS2 cargo proteins. Biochemical characterization of the human ortholog revealed that the protein interacts with a trimeric PEX5/PEX7/PTS2 complex and its presence in a cell-free peroxisomal in vitro import assay potently blocked the association of PEX7 with

peroxisomes and the peroxisomal import of pre-thiolase (a PTS2 protein). Altogether, the data suggest that this conserved protein is a novel peroxin, PEX39, with a specific role in the PTS2-mediated protein import pathway.

Session 2: Peroxisome dynamics

Chairs: Celien Lismont, KU Leuven, Belgium and Suzan Kors, University of Exeter, UK

In the second session, six talks on peroxisome dynamics were presented, covering peroxisome inheritance, division, and membrane contact sites, including diverse regulation mechanisms.

Hien Bui (Pekka Katajisto's laboratory, Institute of Biotechnology, HiLIFE, University of Helsinki, Finland) started the session with an interesting talk on the development of a mouse model and labeling technique that allow following distinct age classes of peroxisomes during stem cell asymmetric divisions. Using this approach, she found that asymmetrically dividing stem cells segregate their old peroxisomes to the daughter cells that will become the new stem cell, both in vitro, in mammary epithelial basal cells (MECs), and in vivo, in epidermal stem cells (EpSCs). **Triana Amen** (Gisou van der Goot's laboratory, Swiss Federal Institute of Technology (EPFL), Switzerland) addressed the regulation of peroxisome biogenesis. She used a small molecule kinase inhibitor screen in CRISPR/Cas9 PMP70-GFP human cells to identify hitherto unknown positive and negative regulators of peroxisome abundance. Among the identified kinases, protein kinase C (PKC) induced peroxisome division through interaction with the peroxisomal membrane and PEX11β in human cells, including neurons, and regulated the metabolic control of peroxisome abundance. **Elena Bittner** (Johannes Freitag's laboratory, Philipps-University Marburg, Germany) shared her latest findings involving proteins with competing targeting signals for peroxisomes and other organelles. She proposed that the sorting of several of these proteins occurs at organellar contact sites and regulates the extent of these organelle proximities. **Ruth Carmichael** (Michael Schrader's laboratory, University of Exeter, UK) discussed recent work from the group identifying a novel mechanism of peroxisome division in mammals (Schrader et al. 2022). Previously, the membrane adaptor MFF, which recruits the fission GTPase DRP1 to the peroxisomal membrane, was thought to be essential for the growth and division pathway of peroxisome proliferation. Using fibroblasts from MFF-deficient patients as a model, which display hyper-elongated peroxisomes, they showed that overexpression of the "master regulator" of peroxisome division, PEX11β, could induce these peroxisomes to divide, thereby bypassing MFF. This required another DRP1 recruitment factor, FIS1, and thus represented a novel pathway driving

peroxisome fission that was MFF independent and PEX11β/FIS1 dependent.

The identification and characterization of inter-organelle communication is currently a priority in cell biology. **Beatriz Silva** (Michael Schrader's laboratory, University of Exeter, UK) presented her work on the identification of a potential communication route between peroxisomes and the nucleus in mammalian cells. In a previous work, the group identified ACBD4 and ACBD5, which bind acyl-CoA fatty acids via their ACB domains, as peroxisomal membrane proteins required for forming peroxisome–ER membrane contact sites (Costello et al. 2017). Beatriz's further characterization of ACBD4 has identified another isoform dually localized to both the nucleus and peroxisomes. This may represent a novel communication pathway integrating peroxisome–ER contacts and lipid metabolism with nuclear functions. **Chloe Bolton** (Joe Costello's laboratory, University of Exeter, UK) presented her progress on a potential regulatory mechanism that controls these peroxisome–ER membrane contact sites via modulation of the interaction between peroxisomal protein ACBD5 and ER-resident VAP proteins. She explored the presence of a phosphodegron site in ACBD5 and the role that this may play in recruiting the ubiquitin machinery to the protein, resulting in its eventual demise via the proteasome degradation pathway. The impact this may have on peroxisome–ER contacts, how this affects peroxisome dynamics, and its potential to disrupt lipid exchange were also discussed.

Session 3: Peroxisome omics

Chairs: Joseph Costello, University of Exeter, UK and **Tony Rodrigues, Universidade do Porto, Portugal**

The identification of new peroxisomal proteins has been a task that has occupied researchers in the field for a number of decades, and it would be tempting to assume that the vast majority of the key peroxisomal proteins have already been identified. However, the findings presented during this session suggested that there are many more important peroxisomal proteins still to characterize, as well as a whole set of proteins that are likely shared with other organelles. The five talks in this session covered the identification and characterization of peroxisomal proteins from humans, mouse, *S. cerevisiae*, *Hansenula polymorpha*, and pepper fruit, using a variety of different approaches.

Maya Schuldiner's research group has used screening approaches to uncover many peroxisomal proteins in the yeast *S. cerevisiae*. **Lior Peer** (Maya Schuldiner's laboratory, Weizmann Institute of Science, Israel) presented their latest work using a collection of strains, each expressing a C-terminal fusion of one yeast protein and the fluorescent protein mNeonGreen on the background of a genomically integrated peroxisomal marker. They then imaged these strains to find cases of colocalization and uncovered 11 proteins that were not observed in peroxisomes before. By defining their subperoxisomal localization, they found that many are peroxisomal membrane proteins, which are dually localized to both peroxisomes and mitochondria. The remaining proteins exhibited a filament-like phenotype in close proximity to peroxisomes, implying a novel function of peroxisomes as a polymerization platform. They suggested that this work, alongside previous efforts, now enables them to define a near-complete peroxi-ome (e.g., peroxisomal proteome) in yeast.

For a detailed understanding of peroxisomal functions, dynamics, and crosstalk with other organelles, knowledge of the complete peroxisomal proteome is essential. **Hirakjyoti Das** (Bettina Warscheid's laboratory, University of Würzburg, Germany) used a spatial proteomics approach based on biochemical fractionation combined with quantitative mass spectrometry, to profile the abundance and distribution of about 10,000 proteins across different subcellular fractions in human cells. They used machine-learning models to assign proteins to distinct subcellular niches and, thereby, define the peroxisomal protein compendium including multilocalized proteins. Through a comparative spatial proteomics approach, they further verified peroxisomal protein localization of newly discovered candidate proteins. These results provide a first high confidence draft of the human peroxisomal proteome comprising more than 180 proteins, of which at least 50 proteins have not been linked to peroxisomes before. **Tjasa Kosir** (Ida van der Klei's laboratory, University of Groningen, The Netherlands) used a different approach to identify peroxisomal proteins, employing proximity labeling in *H. polymorpha* to reveal novel interactors of PEX3. PEX3 is crucial for multiple peroxisome-related processes and can recruit several proteins to peroxisomes. To identify novel PEX3-related functions, they used a *H. polymorpha* strain producing PEX3-TurboID grown at peroxisome-inducing conditions and exposed to biotin. Biotinylated proteins were isolated and analyzed with mass spectrometry. Around 100 highly significant protein hits were obtained, of which around 25% were known PEX3 interactors. Colocalization studies confirmed at least two of these PEX3 interactors as novel peroxisomal proteins and these proteins are currently being analyzed for their function in peroxisome biology. **Öznur Singin** (Markus Islinger's laboratory, Heidelberg University, Germany) next described their work to reassess the proteome of mouse liver peroxisomes. Here, the group employed a quantitative sequential window acquisition of all theoretical mass spectra (SWATH–MS) approach to identify peroxisomal or peroxisome-associated proteins in mouse liver. In total, 203 proteins were significantly enriched from differently obtained peroxisome-enriched fractions. Among those, 41% were

classified as known peroxisomal proteins, while 14% were assigned to mitochondria, 10% to microsomes, and 2% to lysosomes. Some of the novel candidates (e.g., HTATIP2, PAFAH2, or ADE2) contain potential peroxisome targeting sequences, suggesting import into the peroxisome matrix. One novel candidate, OCIA domain-containing protein 1 (OCIAD-1), was further characterized and described to be involved in regulating mitochondrial fission/fusion. Hence, OCIAD-1 may serve as a shared component of the protein machinery controlling peroxisome and mitochondrial dynamics, in a similar way to other well-established shared components such as FIS1 and MFF. Finally, **Salvador González-Gordo** (Francisco Corpas's laboratory, Estación Experimental del Zaidin, CSIC, Spain) presented his work looking at peroxisomes from an unusual source—the sweet pepper. Peroxisomes are known to participate in fruit ripening, but their function in this physiological process is still not well defined. Using sweet pepper fruits at two ripening stages (green and red), peroxisomal protein profiling was analysed by iTRAQ. This approach identified 57 proteins: 36 with a PTS1, 8 with a PTS2, and 5 lacking any type of peptide signal. Most of the proteins were part of the antioxidant and β-oxidation metabolic pathways. Other identified proteins participate in different pathways including purine, sulfur, jasmonic acid, or phenylpropanoid metabolisms. These findings provide new insights into the complex metabolic machinery of peroxisomes in fruit and open new windows of research into the peroxisomal functions during fruit ripening.

Session 4: Peroxisome metabolism

Chairs: Mariana Marques, University of Aveiro, Portugal and **Nicole Linka, Heinrich Heine University, Germany**

The fourth session focused on peroxisome metabolism, from cellular to organismal levels. The first three talks discussed the extent to which perturbations of peroxisomal proteins and their functions can lead to metabolic defects and disease. Furthermore, new insights into the role of peroxisomes in the synthesis of ether phospholipids were presented.

The first speaker, **Santiago José Maya Palacios** (Michael Hoch and Reinhard Bauer's laboratory, University of Bonn, Germany) presented his work on the requirement of the peroxisome biogenesis factor PEX19 for insulin secretion. *Drosophila* peroxisome-deficient PEX19 mutants exhibit multiple metabolic defects, including disturbed insulin signaling. These mutants are unable to secret insulin-like peptides from the insulin producing cells in the brain due to an altered fatty acid profile elicited by peroxisome loss. Click-labeling and lipidomics were applied to show how PEX19

and phosphatidylinositol derivatives contribute to vesicle trafficking and neuropeptide secretion in *Drosophila melanogaster* neurons. Next, **Stephanie Makdissi** (Francesca Di Cara's laboratory, Dalhousie University, Canada) showed how peroxisome metabolism regulates the diet–gut–brain axis leading to neurodegeneration. Peroxisomes are essential regulators of gut lipid metabolism and microbiota, with an implicated role in neuroinflammatory diseases by modulation of the gut–brain axis. In vivo *Drosophila melanogaster* models with mild enterocyte-specific peroxisomal mutations (*Mex > PEX5*-i) elicit behavioral changes and neuroinflammation. These neuro-phenotypes correspond to misregulated gene expression and neuropeptide secretion in the gut. To identify the etiologies and a peroxisome-dependent pathway of neuroinflammation in the gut–brain axis, we must investigate how dietary nutrient metabolism and gut microbiota populations are affected and contribute to this gene misregulation in the gut. **Lingxiao Chen** (Nancy Braverman's laboratory, McGill University, Canada) talked about systemic lipid deficiency and liver pathophysiology in a PEX1-G844D mouse model of mild Zellweger Spectrum Disorder (ZSD). These mice showed hypoglycemia, hypotriglyceridemia and hypoinsulinemia. The underlying pathophysiology suggested a cycle of hypoglycemia–hypoinsulinemia-reduced hepatic lipogenesis, causing systemic lipid deficiency observed in this mouse model and ZSD patients. In addition, a dysregulated hepatic fatty acid transport led to hepato-steatosis. This pathway analysis suggests a novel therapeutic strategy to intervene in the hepatic fatty acid metabolism. Lastly, **Serhii Chornyi** (Hans R. Waterham's laboratory, University of Amsterdam, The Netherlands) discussed the role of peroxisomal β-oxidation and ABC transporters in ether phospholipid synthesis. The essential precursors of the ether phospholipids in humans are synthesized by peroxisomal enzymes during the de novo ether phospholipid pathway. By means of CRISPR/Cas9 genome editing of HeLa cells, cell lines with selective deletions of single or combinations of genes were generated and characterized. Long-chain acyl-CoAs were found to be required for the first step of ether phospholipid synthesis and their import into peroxisomes is mediated by the ABCD proteins. Also, in addition to being imported from the cytosol, these acyl-CoAs can be produced intra-peroxisomally by chain shortening of CoA esters of very long-chain fatty acids via β-oxidation. These results show that peroxisomal β-oxidation and ABCD proteins play a crucial role in the de novo ether phospholipid synthesis.

Session 5: Peroxisomes in health and disease

Chairs: Catherine Argyriou, McGill University, Canada and **Isabelle Weinhofer, Medical University of Vienna, Austria**

In the fifth session, four talks highlighted the importance of peroxisomes for human health, and a nonprofit initiative to support peroxisome disease research was presented.

José M. Horcas-Nieto (Barbara Bakker's laboratory, University of Groningen, The Netherlands) developed and characterized a translational organoid model that recapitulates liver-specific metabolic manifestations of malnutrition. This model was used to understand the underlying mechanisms behind peroxisomal and mitochondrial dysfunction in malnutrition, as well as to test different pharmacological approaches. **Hongli Li** (Marc Fransen's laboratory, KU Leuven, Belgium) reported his systematic comparison of the skin fibroblasts derived from a control individual and a patient with deleterious PEX10-p.[H310D/E10Gfs] variants. He found that the PEX10-deficient patient cells exhibit decreased intraperoxisomal glutathione redox potential with increased intramitochondrial H_2O_2 levels, as well as disturbed autophagy. **Daniëlle Swinkels** (Myriam Baes's laboratory, KU Leuven, Belgium) presented the outcome of her study of whether the retinal morphology of *Mfp2* KO mice could be ameliorated by docosahexaenoic acid (DHA) supplementation. She observed that DHA treatment of *Mfp2* KO mice rescued the retinal phenotype at an early age but was unable to prevent retinal demise at later age, most likely due to the cell autonomous function of MFP2 in the retina pigment epithelium. **Ali Tawbeh** (Stéphane Savary's laboratory, Université de Bourgogne, France) reported his findings on understanding the pathophysiology underlying neurodegeneration in X-linked adrenoleukodystrophy (X-ALD). He presented a murine microglial cell model with KO peroxisomal ABC-transporters *Abcd1* and *Abcd2,* or the peroxisomal β-oxidation enzyme *Acox1,* and outlined co-culture experiments with negative impact on neuronal morphology and survival. **Andrew Longenecker** (PBD Project, USA) presented the case of his son Diego, who suffers from *PEX10*-mediated ZSD, a peroxisome biogenesis disorder (PBD) for which he and his wife started a nonprofit called PBD Project (Research@PBDProject.org). Recent work supported by this initiative shows that *PEX10* mutations may inhibit polyubiquitination and removal of PEX5 from the peroxisome membrane, indirectly preventing subsequent rounds of peroxisome import. This suggests that the rate of peroxisome degradation versus turnover may be a determining factor in *PEX10*-mediated ZSD.

Session 6: Peroxisomal homeostasis

Chairs: Sai Kocherlakota, KU Leuven, Belgium and **Victoria Riccio, University of Toronto, Canada**

Session six started off with **Cláudio Figueiredo Costa** (Marc Fransen's laboratory, KU Leuven, Belgium) presenting his findings on GSTK1, the only known peroxisomal glutathione-consuming enzyme. He generated a GSTK1 KO HEK-293 cell line to obtain clues about the potential function of the protein. Basal characterization of KO cells did not reveal major differences in comparison with the control counterparts. However, the recovery of the peroxisomal glutathione redox state after an oxidative insult was significantly delayed in KO cells, indicating that GSTK1 has a role in glutathione homeostasis in peroxisomes. In addition, Costa and colleagues observed that such impairment could be rescued by targeting glutaredoxin-1 to peroxisomes, suggesting that GSTK1 may function as a glutathione-disulfide oxidoreductase. He aims to delineate the molecular mechanisms behind this in his future work. **Akihiro Yamashita's** (Heidi McBride's laboratory, McGill University, Canada) presentation focused on the outcome of silencing LONP2, a peroxisomal Lon protease, in COS-7 cells and U2OS cells. Their results suggest that LONP2 knockdown triggers ubiquitous peroxisomal phenotypes across multiple cell lines, where the extent of global cellular stress responses is dependent on the cell type. Akihiro has also indicated that cholesterol flux between lysosomes and ER was blocked, and the increased cholesteryl esters were trapped in lysosomes upon silencing of LONP2. **Ismaila Francis Yusuf's** (Ralf Erdmann's laboratory, Ruhr University, Bochum, Germany) presentation focused on the quality control of the peroxisomal import receptor PEX5p in *S. cerevisiae*. First, he aimed to identify the protein that facilitates PEX5p extraction and mediates its proteasomal degradation when the receptor recycling machinery is defective, as in PEX1 mutant cells. To this end, he showed that PEX5p is rapidly degraded in PEX1 deficient cells during the exponential growth phase of *S. cerevisiae*, in contrast to the observation at the stationary growth phase. Additionally, he demonstrated that PEX5p degradation in PEX1 mutant cells requires the peroxisomal membrane and is not a result of pexophagy. Finally, he used a CDC48 conditional KO strain under the control of the Gal1 promoter and showed that the knockdown of CDC48 in PEX1 mutant cells stabilized PEX5p, implying that Cdc48p facilitates PEX5p degradation when its extraction by PEX1p is blocked. He further investigated whether Cdc48p is required for the degradation of other peroxins, such as PEX18p and PEX13p. However, the knockdown of CDC48 did not terminate the degradation of PEX18p and PEX13p, thus suggesting the presence of a different quality control pathway at the peroxisome that targets these peroxins.

Moving on to autophagy, while numerous selective autophagy pathways occur in parallel within the cell, **Kyla Germain** (Peter Kim's laboratory, Hospital for Sick Children, Canada) aimed to identify whether the degradation of one organelle/substrate impacts that of another. Kyla and colleagues tested the effects of upregulated pexophagy (peroxisome degradation) on the turnover of other autophagy

substrates, and report that upregulated pexophagy limits the autophagic degradation of both damaged mitochondria and protein aggregates. They further examined the effects of aberrant pexophagy in cell and mouse models of ZSD to determine how it may contribute to liver pathologies associated with ZSD.

The following talks provided an update on peroxisomal calcium dynamics, of which very little is known. With a fluorescence resonance energy transfer (FRET)-based genetically encoded calcium sensor targeted to peroxisomes, **Julia Kalinowski** (Sven Thoms's laboratory, University of Bielefeld, Medical School EWL, Germany) found that calcium levels follow cytosolic levels in HeLa cells and cardiomyocytes when calcium is released from intracellular stores and upon uptake by store-operated calcium entry (SOCE) (Sargsyan et al. 2021). Peroxisomes may constitute a buffer compartment that alleviates conditions of calcium overload (Sargsyan et al. 2022). To investigate the mechanism of calcium uptake into peroxisomes, Kalinowski and colleagues conducted a candidate approach and inactivated various peroxisomal membrane proteins. They identified PXMP2 as a potential peroxisomal calcium channel. Finally, **Maria João Ferreira** (Jorge Azevedo's laboratory, Universidade do Porto, Portugal) described a post-nuclear supernatant-based in vitro strategy particularly suited to examine the redox dynamics of peroxisomes. The new strategy comprises a first step in which peroxisomes are equipped in vitro with a specific and sensitive glutathione redox sensor, followed by a second step in which the redox status of the cytosol is rapidly changed by adding a bolus of reduced or oxidized glutathione (GSH and GSSG, respectively). After showing that the redox sensor is properly imported and folded in the peroxisomal matrix, Maria presented redox kinetics data. The findings provide a new perspective on glutathione biology in the mammalian peroxisomal matrix.

Session 7: Peroxisomes and pathogens

Chairs: Vanessa Ferreira, University of Aveiro, Portugal and Francesca Di Cara, Dalhousie University, Canada

There is now substantial evidence that peroxisomes actively contribute to the host's immune signaling and are hijacked by pathogens to evade host-mediated immune responses. Understanding how peroxisomes participate in host–pathogen interactions is an exciting up-and-coming area of investigation. The findings, presented in this session by talented young researchers, clearly show the increasing contribution that the study of peroxisomal signaling is bringing to the fields of virology, microbiology, parasitology, and immunology.

Enrica Pellegrino (Maximiliano Gutierrez' laboratory, Francis Crick Institute, United Kingdom) showed that infection of human macrophages with *Mycobacterium tuberculosis* (Mtb) triggered an increase in the number of peroxisomes and peroxisome-derived reactive oxygen species (ROS). Moreover, macrophages lacking peroxisomes were shown to have a lower capacity to restrict Mtb infection due to a decreased production of ROS. This study also uncovers a new role for peroxisomes in the host response to Mtb. **Valeria Napolitano**, (Grzegorz Popowicz's laboratory, Helmholtz Zentrum München, Germany) demonstrated that glycosomal protein import is essential for the survival of *Trypanosoma*. In fact, small molecule inhibitors of protein–protein interactions (PEX14 and PEX5), which play a crucial role in the glycosomal protein import, were identified. These results provide ground for the development of new therapies against trypanosomiases.

Viruses have developed several ways to evade the host cell's response to infection. The mechanism of RNAi is the primary defense mechanism of plants against viral pathogens. The cysteine-rich 15 kDa protein (P15) of the peanut clump virus can sequester siRNAs into peroxisomes via its C-terminal PTS1 as a strategy to survive the host immune defense. **Stefan Wirling** (Sigrun Reumann's laboratory, University of Hamburg, Germany) found that this mechanism is conserved in other genera of plant viruses. Using a subcellular targeting analysis approach, Stefan showed that multiple viral proteins are targeted to peroxisomes. Furthermore, the author expressed the P15 protein in *E. coli* for biophysical characterization and determined the binding affinity to siRNAs. **Ana Rita Ferreira** (Daniela Ribeiro's laboratory, University of Aveiro, Portugal) presented her latest results on the evasion mechanisms employed by the human cytomegalovirus (HCMV) to hinder the cellular antiviral signaling at peroxisomes and mitochondria. Ferreira and colleagues proposed a model in which HCMV's protein vMIA interacts with the peroxisomal biogenesis factor PEX19 at the cytoplasm to travel to peroxisomes, where it interacts with the antiviral signaling protein MAVS. This interaction interferes, in a MFF-dependent manner, with the formation of MAVS oligomers, and inhibits the consequent activation of the downstream antiviral signaling (Ferreira et al. 2022a). Their results show the relevance of peroxisomes as platforms for antiviral signaling against HCMV and unravel specific molecular mechanisms that may be further explored as targets for antiviral therapy.

All the presented research highlights the pivotal role of peroxisomes as regulators of cellular signalling towards pathogens, to aid the host's survival upon infection. These discoveries are paving the way towards the potential role of peroxisomes as a therapeutic target for drug discovery against various pathogens.

Closing of the meeting

In the closing session, all participants were invited to vote for the best oral and poster presentations, which were awarded to Kyla Germain and Victoria Riccio, respectively, both from Peter Kim's lab (University of Toronto, Canada). After farewell and thankful words from the hosts, Daniela Ribeiro and Jorge Azevedo, it was announced that the 9th OEPM will be organized by Ralf Erdmann and Michael Schrader in Costa Brava (Spain) in 2024.

The 8th OEPM was a long-awaited get together, due to the COVID-19 restrictions. The peroxisome research community showed up in force, with researchers finally having the opportunity to meet in person again. It was a very successful meeting, rich in oral and poster presentations, and timely discussions and exchanges of ideas. It was an amazing meeting where new collaborations were established, and old ones were reinforced.

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