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# Mitochondria as emergency landing for abandoned peroxins

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**Zellweger spectrum disorder (ZSD) is the most severe peroxisomal biogenesis disorder (PBD). Why ZSD patients not only loose functional peroxisomes but also present with severe mitochondrial dysfunction was a long-standing mystery. In this issue, Nuebel *et al* (2021) identified that loss of peroxisomes leads to re-routing of peroxisomal proteins to mitochondria, thereby impairing mitochondrial structure and function. The findings provide the first molecular understanding of the mitochondrial-peroxisomal link in ZSD.**

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See also: **E Nuebel *et al*** (October 2021)

Peroxisomes are small, ubiquitous organelles that harbor essential catabolic and anabolic pathways, and peroxisomal defects cause a large variety of disorders. Peroxisomal biogenesis disorders (PBDs) are derived from mutations in *PEX* genes encoding the peroxins, which are required for peroxisomal biogenesis. Consequently, PDBs can present with decreased peroxisome biogenesis and abundance, non-functional peroxisomes, or complete loss of the organelle. The most severe PBD is the Zellweger spectrum disorder (ZSD) that manifests soon after birth and leads to death in early childhood affecting several organs, like brain, liver, or kidney (Goldfischer *et al*, 1973; Saraya *et al*, 2010; Walter & Erdmann, 2019; Honsho *et al*, 2020). On a molecular level, ZSD patients represent with non-functional peroxisome but also with mitochondrial defects like decreased respiration or aberrant mitochondrial morphology. However, the molecular link joining primary defects in peroxisomal biogenesis and dysfunctional mitochondria remained elusive. In this issue of EMBO reports, Nuebel *et al* (2021) uncover this missing linking and present the biochemical basis of mitochondrial dysfunction in ZSD.

Nuebel *et al* (2021) investigate the molecular consequences that the loss of peroxisomes has for peroxisomal proteins by employing elegant experiments in the model system *Saccharomyces cerevisiae*. Deletion of Pex3 or Pex19, peroxins required for import of peroxisomal membrane proteins, results in loss of functional peroxisomes; however, transcription and translation of peroxin-encoding genes was maintained. What happens now to the peroxins when their target organelle is missing? In a proteomics approach using purified mitochondria, Nuebel *et al* (2021) identify mitochondria as the prime destination for re-routing of peroxisomal proteins. They detect accumulation of several peroxins in the mitochondrial fraction of *pex19Δmsp1Δ* cells, that lack outer membrane quality control by Msp1/ATAD1 (Chen *et al*, 2014; Okreglak & Walter, 2014), and even detect peroxisomal matrix proteins indicating that a substantial part of the peroxisomal proteome is re-routed to mitochondria when their actual target, the peroxisomes, are absent. As several subunits of the peroxisomal import machinery were detected in the mitochondrial fraction, e.g., the docking complex (Pex13, Pex14, Pex17), the authors next assessed the capability of these subunits to assemble into a functional import complex in this non-native organelle. Blue-native PAGE analysis identified a 180 kDa complex and subsequent MS analysis confirmed the presence of the docking complex and several other subunits of the peroxisomal importomer in mitochondria. The abundance of the peroxins in the mitochondrial membrane was strongly increasing upon lack of Msp1.

The authors then proceeded with analysis of fibroblasts from ZSD patients, which they engineer to recapitulate their experimental yeast set-up. Intriguingly, lack of PEX3 resulted in localization of a peroxisomal marker protein (PEX13-GFP) to mitochondria even in the presence of the Msp1 homologue ATAD1. While significant accumulation of peroxisomal proteins in yeast mitochondria was requiring deletion of Msp1, only overexpression of ATAD1 reduced mitochondrial peroxin localization. This might be due to PEX13-GFP overexpression after lentiviral transfection or could indicate a lower specificity or limited capacity of ATAD1 toward peroxins in human cells. While the abundance of endogenous PEX proteins in mitochondria in the ZSD cell lines awaits further investigation, a clear link to mitochondrial dysfunction is already revealed: aberrant mitochondrial morphology observed in ZSD cells improved by overexpression of ATAD1. Similarly, mitochondrial respiration was rescued by increased ATAD1 expression, but worsened upon lack of ATAD1. While ATAD1, like Msp1, has a protective effect for mitochondria, endogenous levels of ATAD1 are not sufficient to counteract the deleterious accumulation of peroxisomal proteins on mitochondria.

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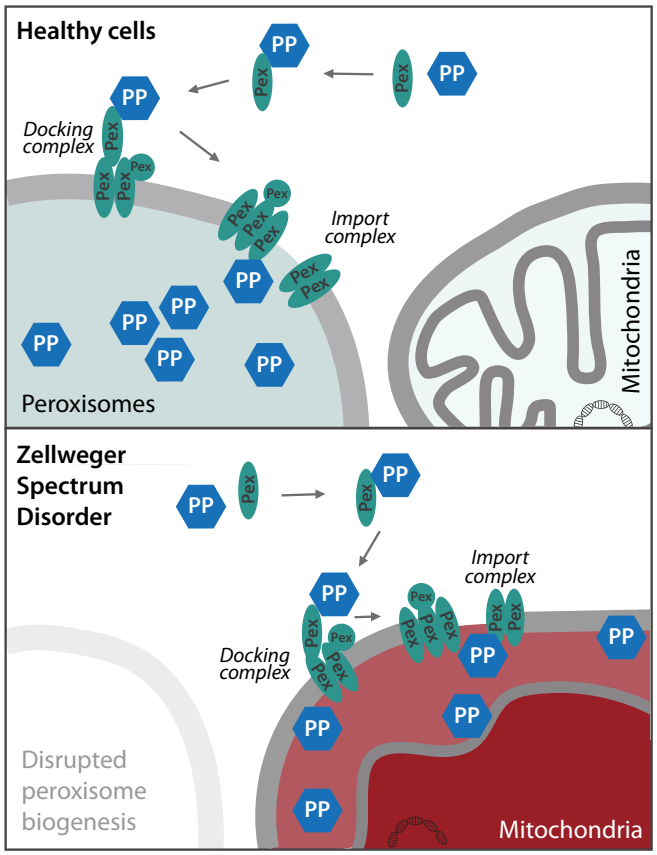


**Figure 1. Re-routing peroxisomal proteins to mitochondria in Zellweger Spectrum Disorder.**

In healthy cells, the different peroxins (Pex) mediate transfer of fully synthesized peroxisomal proteins (PP) from the cytosol to the peroxisomal import machinery where they are imported to build and maintain functional peroxisomes. In Zellweger spectrum disorder, peroxisomes are lost and peroxins are re-routed to the mitochondrial outer membrane and assembled into a functional import machinery. This allows import of further peroxisomal precursor proteins into mitochondria and eventually leads to impaired mitochondrial structure and function.

The identification of the molecular basis of mitochondrial dysfunction in ZSD by Nuebel *et al* (2021) allows for the first time the distinction of primary effects caused by missing peroxisomes and secondary consequences. Transfer of these new insights into Zellweger mouse models will allow investigation of possible protective effects of ATAD1 overexpression on organismal health. But also on the molecular level, the findings have opened up new and highly exciting research topics: Does the docking and importomer complex form *de novo* on mitochondria upon lack of peroxisomes or is there constitutive mislocalization of peroxisomal proteins to mitochondria? Ribosome profiling revealed that several peroxisomal proteins are synthesized in the vicinity of peroxisomes (Zipor *et al*, 2009), and maybe, this localized translation is a mean to favor peroxisomal targeting, but shifts toward mitochondria in the absence of peroxisomes. Does this mitochondrial re-routing increase and overburden the mitochondrial quality control system upon loss of peroxisomes? This would be especially interesting to investigate under physiological conditions of rapid peroxisomal expansion (e.g., growth on oleic acid) and could reveal if physiological scenarios exist in which peroxisomal proteins are re-routed and functional in mitochondria. Furthermore, the assembly of a functional import machinery in a non-native organelle is an intriguing observation that raises several questions: Proteins can cross the peroxisomal membrane in a folded or even oligomeric state, while mitochondrial protein import occurs in an unfolded conformation. Import of soluble peroxisomal proteins into mitochondria implies that the functional docking complex and importomer is formed at least transiently during the import process, while the entire import route requires a cyclic assembly/disassembly of these machineries. What consequences do this transient pore formation have for the mitochondrial outer membrane integrity and maintenance of the critical redox environment in the intermembrane space? Finally, the observed mitochondrial defects in ZSD are severe and might not be only caused by accumulation of additional alien proteins as mitochondria are equipped with several proteases and can efficiently degrade mislocalized proteins (Szczepanowska & Trifunovic, 2021). The consequences downstream of peroxin importomer formation and subsequent mislocalization of peroxisomal proteins inside mitochondria therefore await further investigation.

Taken together, the publication by Nuebel *et al* (2021) identifies the molecular basis for mitochondrial dysfunction in ZSD and their study is a further, excellent example of how unicellular model organisms like yeast can be exploited to gain molecular insight into conserved processes that are highly relevant to understand human disease pathogenesis (Fig 1).

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