

# Transient complex peroxisomal interactions

## A new facet of peroxisome dynamics in mammalian cells

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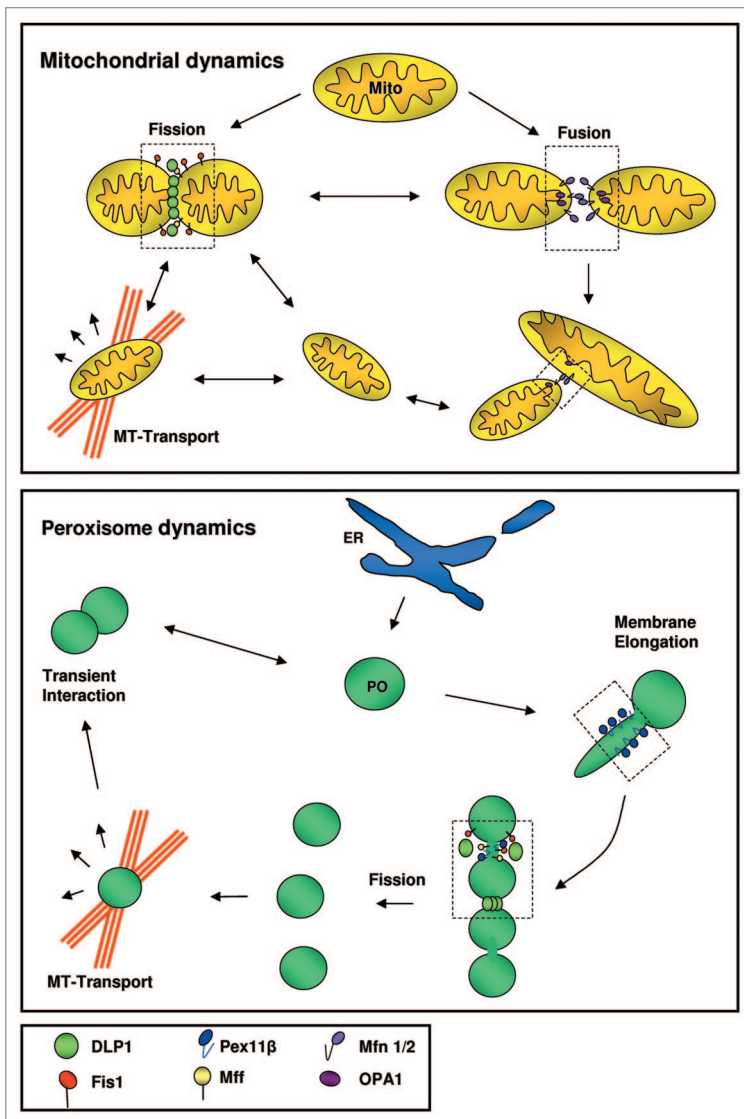
Mitochondria and peroxisomes are ubiquitous subcellular organelles that fulfill essential metabolic functions, rendering them indispensable for human development and health. Both are highly dynamic organelles that can undergo remarkable changes in morphology and number to accomplish cellular needs. While mitochondrial dynamics are also regulated by frequent fusion events, the fusion of mature peroxisomes in mammalian cells remained a matter of debate. In our recent study, we clarified systematically that there is no complete fusion of mature peroxisomes analogous to mitochondria. Moreover, in contrast to key division components such as DLP1, Fis1 or Mff, mitochondrial fusion proteins were not localized to peroxisomes. However, we discovered and characterized novel transient, complex interactions between individual peroxisomes which may contribute to the homogenization of the often heterogeneous peroxisomal compartment, e.g., by distribution of metabolites, signals or other “molecular information” via interperoxisomal contact sites.

Mitochondria and peroxisomes are ubiquitous subcellular organelles that fulfill essential metabolic functions, rendering them indispensable for human development and health. Although mitochondria are often reduced to being “the powerhouse of the cell,” other essential mitochondrial metabolic processes are performed in close co-operation with peroxisomes in mammalian cells (e.g., the  $\beta$ -oxidation of fatty acids, metabolism of reactive oxygen species (ROS), anti-viral signaling).<sup>1–4</sup> Moreover, mitochondria and peroxisomes are highly dynamic organelles that can undergo drastic changes in morphology and number to accomplish cellular needs (Fig. 1). Mitochondrial dynamics are regulated by a combination of frequent fusion and fission events which serve to homogenize the compartment in regard to lipid, protein and mtDNA composition.<sup>5,6</sup> Furthermore, proper distribution of mitochondria throughout the cell is mediated by microtubule-dependent movements, which are important for axonal development and neuronal survival.<sup>7,8</sup> Peroxisome dynamics are similarly regulated by organelle fission and microtubule-dependent distribution.<sup>9,10</sup> Growth and division of the peroxisomal compartment follow morphologically well-defined steps of membrane deformation/elongation, constriction and final fission<sup>11</sup> (Fig. 1). Interestingly, peroxisomes and mitochondria share key components of their division machinery: DLP1, a large GTPase mediating final membrane scission is recruited to both organelles via its membrane receptors Fis1 and Mff, which as well localize to

peroxisomes and mitochondria<sup>9,11–16</sup> (Fig. 1). However, the question whether peroxisome fusion also contributes to peroxisome dynamics in mammalian cells remained a matter of debate. Fusion of pre-peroxisomal vesicular structures has however been implicated in the biogenesis of peroxisomes in yeast.<sup>17,18</sup>

We recently addressed this question by employing an *in vivo* fusion assay based on the co-cultivation of mammalian CHO cells stably expressing either red or green fluorescent peroxisomal (matrix or membrane) proteins. Subsequently, hybridoma cells were generated and screened for an intermixing of red and green marker proteins (potentially resulting in the appearance of “yellow” peroxisomes due to fluorescent marker overlay) after further incubation at 37°C (to guarantee optimal cellular conditions/peroxisome motility) (1 – 6 h) by a combination of epifluorescence microscopy (in fixed cells) as well as spinning disk confocal microscopy and live cell imaging. Successful shut-down of protein biosynthesis in the course of the experiment was verified by pulse-chase labeling with S<sup>35</sup>-methionine excluding false positives by protein import into peroxisomes. Successful fusion of mitochondria served as a positive control. In line with previous observations in yeast, plant and mammalian cells,<sup>19–21</sup> a complete peroxisomal fusion mechanism analogous to mitochondria could be excluded. Moreover, evidence was provided by combining overexpression studies, epifluorescence microscopy and immunoblotting that although peroxisomes and mitochondria share

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**Figure 1.** Comparison of mitochondrial and peroxisomal dynamics. Mitochondrial dynamics (upper panel) are regulated by a combination of frequent fusion and fission events that serve to homogenize the mitochondrial compartment. Mitochondrial fission is mediated by the action of Mff (yellow), Fis1 (red) and DLP1 (green), while fusion is concerted by the action of mitofusins (Mfn, purple) and OPA1 (violet). Note that fused mitochondria might further interact and fuse to form mitochondrial networks (lower right corner). Transport of mitochondria throughout the cell is facilitated by microtubule-dependent movements. Note that individual mitochondria continuously engage in cycles of fusion and fission. Peroxisomal dynamics (lower panel) are regulated by peroxisomal growth and division: unlike mitochondria, formation of tubular peroxisomal structures is facilitated by inherent membrane elongation mediated by Pex11 proteins (e.g., Pex11 $\beta$ , blue).<sup>36</sup> After constriction, fission into spherical organelles is mediated by the same key components required for mitochondrial fission. Long-range transport of peroxisomes is mediated by the microtubule cytoskeleton. Unlike mitochondria, peroxisomes do not fuse and do not share key mitochondrial fusion proteins. However, peroxisomes are engaged in transient interactions that, in combination with long-range transport, may potentially serve to homogenize the peroxisomal compartment. Furthermore, peroxisomes can form de novo from the ER under special conditions, which is not possible for mitochondria.

components of their division machinery, mitochondrial fusion proteins (e.g., Mfn1, Mfn2, Opa1) do not contribute to peroxisome dynamics (Fig. 1).

However, in depth-analyses of CHO hybridoma cells by deconvolution microscopy and live cell imaging revealed for the first time that individual red and green peroxisomes (a subpopulation of around 4% at all time points examined) were engaged in several transient, but vivid and long-term contacts, some of which extended over the total observation time (~20 min) (Fig. 2). Detailed mathematical analysis of the duration of those interaction events suggested that the distribution of long-term contacts displays so-called power law behavior,<sup>22-27</sup> as the number of long-term peroxisomal contacts is substantially larger than could be expected from an exponential distribution which would in turn indicate random events. Interestingly, power law distributions in biological processes are indicative of the existence of intricate dynamics originating from diverse, and yet specific mechanisms, suggesting that peroxisome interactions are more complex than previously assumed. Thus, a new dynamic behavior of peroxisomes was characterized in our study.

Still, how may interactions between only a subpopulation of peroxisomes potentially contribute to peroxisome dynamics? Using a simple computational model, we demonstrated that a combination of ATP-driven peroxisome movement (performed by ~15% of the peroxisome population)<sup>10,28,29</sup> and subsequent formation of inter-peroxisomal contacts may potentially contribute to e.g., the homogenization of the often heterogeneous peroxisomal compartment (e.g., by distribution of metabolites, signals or other “molecular information”) in the course of 1–3 h (Fig. 1). Interestingly, our model points to a relationship between the percentage of fast moving peroxisomes, energy consumption and the mixing time of different peroxisome populations within a cell. Hence, our studies indicate for the first time that around 15% of peroxisomes engage in long-range microtubule-dependent movements due to a potential evolutionary optimization process aiming at the homogenization of the peroxisomal compartment at low energy costs. Strikingly, our model is consistent with the experimental observation shortly after hybridoma formation that previously separated red and green peroxisome populations acquire a uniform, interspersed distribution within the cell. Microtubule-driven fast movements of peroxisomes and subsequent transient contacts might thus contribute to equilibrate peroxisome pools throughout the cell.

But in what respect may peroxisomal populations require homogenization and which components might be exchanged? As peroxisomes are very heterogeneous in terms of density, protein composition and import competence in different species, organs and cells as well as within the same cell,<sup>30-35</sup> an exchange of metabolic information might be required. Our initial experiments do not support the exchange of protein markers, but a close apposition of peroxisomes

might favor an exchange of certain metabolites between heterogeneous organelles. Nonetheless, an increase in heterogeneity among different peroxisome populations by manipulating ROS and fatty acid levels did not result in an increase of peroxisome interactions, thus questioning the exchange of metabolites by inter-peroxisomal contacts. Alternatively, the transient complex peroxisomal interactions might be part of a “signaling system” to sense the state and/or distribution of the peroxisomal populations within the cell. Although the physiological significance of the inter-peroxisomal contacts requires further investigation, the identification of those complex interactions adds a novel, interesting twist to peroxisome dynamics.

#### Disclosure of Potential Conflicts of Interest

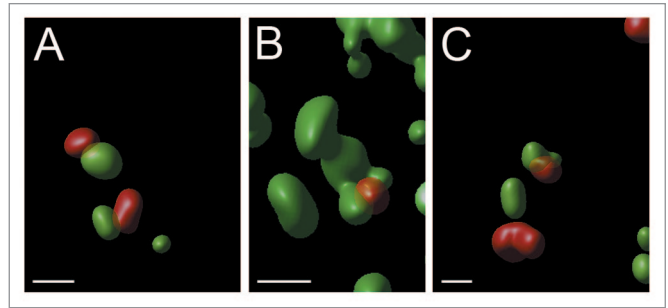
No potential conflicts of interest were disclosed.

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**Figure 2.** Deconvolution microscopy of red and green peroxisomes in hybridoma cells, which are engaged in transient but close contacts. (A-C) A selection of cut-outs from different hybridoma cells is shown. GFP-PTS1 (green), DsRed-PTS1 (red). Differentially labeled CHO cells were subjected to an in vivo peroxisomal fusion assay. Cells were then fixed and mounted for confocal microscopy using a Leica TCS SP2 AOBs confocal microscope equipped with a 100x objective. Using the 488 and 543 nm laser lines, z-stacks were generated (settings: 8x zoom) using the optimal number of slices suggested by the program (Leica Confocal Software). Oversaturation of signals (and thus overinterpretation of peroxisome radii) was avoided by adjusting of respective photomultipliers. Image deconvolution was performed using Huygens Professional Software (Scientific Volume Imaging, Hilversum, The Netherlands). Using the 3D images generated by the program, interacting peroxisomes were assessed for colocalization of signals and mean distance between objects using the tools “colocalisation parameters” and “distance to reference objects,” respectively. Bars, 0.5  $\mu$ m.

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