Communicative & Integrative Biology 5:6, 534–537; November/December 2012 © 2012 Landes Bioscience

Transient complex peroxisomal interactions
A new facet of peroxisome dynamics in mammalian cells

Nina A. Bonekamp[1] and Michael Schrader[1,2,*]

[1]Centre for Cell Biology & Dept. of Biology; University of Aveiro; Campus Universitário de Santiago; Portugal; [2]College of Life and Environmental Sciences, Biosciences; University of Exeter; Geoffrey Pope Building; Exeter, UK

Keywords: peroxisomes, mitochondria, fusion, organelle dynamics, interaction

Abbreviations: CHO, Chinese Hamster Ovary; DLP1, Dynamin-like protein 1; Fis1, Fission 1; Mff, mitochondrial fission factor; Mfn, mitofusin; OPA1, optic atrophy protein 1

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 β-oxidation of fatty acids, metabolism of reactive oxygen species (ROS), anti-viral signaling).[1-4] 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.[5,6] Furthermore, proper distribution of mitochondria throughout the cell is mediated by microtubule-dependent movements, which are important for axonal development and neuronal survival.[7,8] Peroxisome dynamics are similarly regulated by organelle fission and microtubule-dependent distribution.[9,10] Growth and division of the peroxisomal compartment follow morphologically well-defined steps of membrane deformation/elongation, constriction and final fission[11] (**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[9,11-16] (**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.[17,18]

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^{35}-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,[19-21] 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

*Correspondence to: Michael Schrader; Email: m.schrader@exeter.ac.uk
Submitted: 06/11/12; Revised: 07/13/12; Accepted: 07/16/12
http://dx.doi.org/10.4161/cib.21508

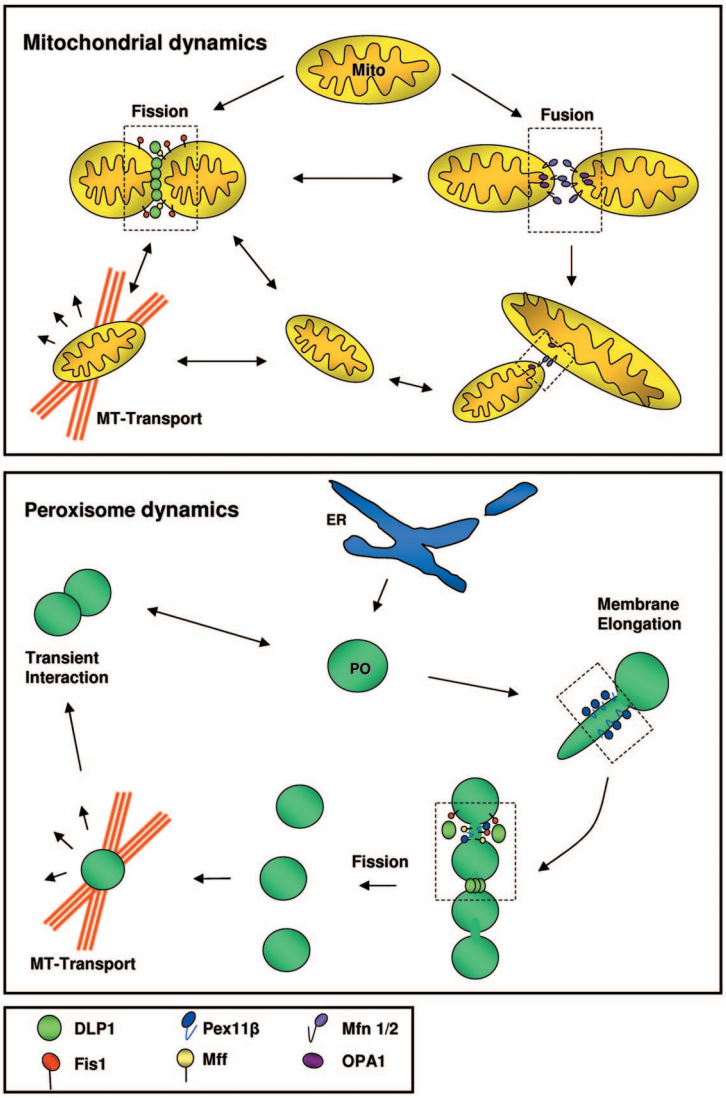

Figure 1. Comparison of mitochondrial and peroxisomal dynamics. Mitochondrial dynamics (upper panel) are regulated by a combination of frequent fusion and fission events that serve to homogenize the mitochondrial compartment. Mitochondrial fission is mediated by the action of Mff (yellow), Fis1 (red) 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., Pex11pβ, blue).[36] 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,[22-27] 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)[10,28,29] 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,[30-35] 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.

Acknowledgments

This work was supported by grants from CRUP/DAAD (A-20/08) and FCT (PTDC/SAU-OSM/103647/2008, SFRH/BD/37647/2007 to N.A.B).

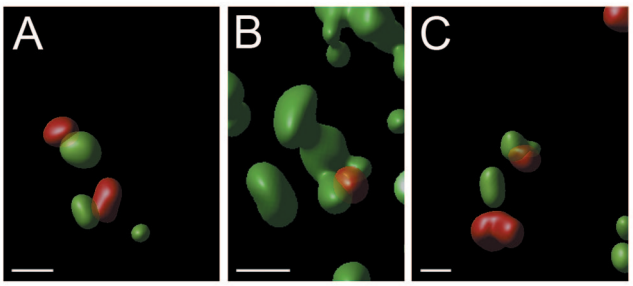

Figure 2. Deconvolution microscopy of red and green peroxisomes in hybridoma cells, which are engaged in transient but close contacts. (A-C) A selection of cut-outs from different hybridoma cells is shown. GFP-PTS1 (green), DsRed-PTS1 (red). Differentially labeled CHO cells were subjected to an in vivo 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, 0.5μm.

References

1. Bonekamp NA, Völkl A, Fahimi HD, Schrader M. Reactive oxygen species and peroxisomes: struggling for balance. Biofactors 2009; 35:346-55; PMID:19459143; http://dx.doi.org/10.1002/biof.48.
2. Dixit E, Boulant S, Zhang Y, Lee AS, Odendall C, Shum B, et al. Peroxisomes are signaling platforms for antiviral innate immunity. Cell 2010; 141:668-81; PMID:20451243; http://dx.doi.org/10.1016/j.cell.2010.04.018.
3. Fransen M, Nordgren M, Wang B, Apanasets O. Role of peroxisomes in ROS/RNS-metabolism: Implications for human disease. Biochim Biophys Acta 2012; 1822:1363-73; PMID:22178243; http://dx.doi.org/10.1016/j.bbadis.2011.12.001.
4. Schrader M, Yoon Y. Mitochondria and peroxisomes: are the 'big brother' and the 'little sister' closer than assumed? Bioessays 2007; 29:1105-14; PMID:17935214; http://dx.doi.org/10.1002/bies.20659.
5. Bereiter-Hahn J, Jendrach M. Mitochondrial dynamics. Int Rev Cell Mol Biol 2010; 284:1-65; PMID:20875628; http://dx.doi.org/10.1016/S1937-6448(10)84001-8.
6. Westermann B. Mitochondrial fusion and fission in cell life and death. Nat Rev Mol Cell Biol 2010; 11:872-84; PMID:21102612; http://dx.doi.org/10.1038/nrm3013.
7. Ishihara N, Nomura M, Jofuku A, Kato H, Suzuki SO, Masuda K, et al. Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. Nat Cell Biol 2009; 11:958-66; PMID:19578372; http://dx.doi.org/10.1038/ncb1907.
8. Wakabayashi J, Zhang Z, Wakabayashi N, Tamura Y, Fukaya M, Kensler TW, et al. The dynamin-related GTPase Drp1 is required for embryonic and brain development in mice. J Cell Biol 2009; 186:805-16; PMID:19752021; http://dx.doi.org/10.1083/jcb.200903065.
9. Schrader M, Fahimi HD. Growth and division of peroxisomes. Int Rev Cytol 2006; 255:237-90; PMID:17178468; http://dx.doi.org/10.1016/S0074-7696(06)55005-3.
10. Schrader M, Thiemann M, Fahimi HD. Peroxisomal motility and interaction with microtubules. Microsc Res Tech 2003; 61:171-8; PMID:12740823; http://dx.doi.org/10.1002/jemt.10326.
11. Schrader M, Bonekamp NA, Islinger M. Fission and proliferation of peroxisomes. Biochim Biophys Acta 2012; 1822:1343-57; PMID:22240198; http://dx.doi.org/10.1016/j.bbadis.2011.12.014.
12. Camões F, Bonekamp NA, Delille HK, Schrader M. Organelle dynamics and dysfunction: A closer link between peroxisomes and mitochondria. J Inherit Metab Dis 2009; 32:163-80; PMID:19067229; http://dx.doi.org/10.1007/s10545-008-1018-3.
13. Gandre-Babbe S, van der Bliek AM. The novel tail-anchored membrane protein Mff controls mitochondrial and peroxisomal fission in mammalian cells. Mol Biol Cell 2008; 19:2402-12; PMID:18353969; http://dx.doi.org/10.1091/mbc.E07-12-1287.
14. Koch A, Thiemann M, Grabenbauer M, Yoon Y, McNiven MA, Schrader M. Dynamin-like protein 1 is involved in peroxisomal fission. J Biol Chem 2003; 278:8597-605; PMID:12499366; http://dx.doi.org/10.1074/jbc.M211761200.
15. Koch A, Yoon Y, Bonekamp NA, McNiven MA, Schrade r M. A role for Fis1 in both mitochondrial and peroxisomal fission in mammalian cells. Mol Biol Cell 2005; 16:5077-86; PMID:16107562; http://dx.doi.org/10.1091/mbc.E05-02-0159.
16. Otera H, Wang C, Cleland MM, Setoguchi K, Yokota S, Youle RJ, et al. Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells. J Cell Biol 2010; 191:1141-58; PMID:21149567; http://dx.doi.org/10.1083/jcb.201007152.
17. Titorenko VI, Rachubinski RA. Peroxisomal membrane fusion requires two AAA family ATPases, Pex1p and Pex6p. J Cell Biol 2000; 150:881-6; PMID:10953011; http://dx.doi.org/10.1083/jcb.150.4.881.
18. van der Zand A, Gent J, Braakman I, Tabak HF. Biochemically distinct vesicles from the endoplasmic reticulum fuse to form peroxisomes. Cell 2012; 149:397-409; PMID:22500805; http://dx.doi.org/10.1016/j.cell.2012.01.054.
19. Arimura S, Yamamoto J, Aida GP, Nakazono M, Tsutsumi N. Frequent fusion and fission of plant mitochondria with unequal nucleoid distribution. Proc Natl Acad Sci U S A 2004; 101:7805-8; PMID:15136720; http://dx.doi.org/10.1073/pnas.0401077101.
20. Huybrechts SJ, Van Veldhoven PP, Brees C, Mannaerts GP, Los GV, Fransen M. Peroxisome dynamics in cultured mammalian cells. Traffic 2009; 10:1722-33; PMID:19719477; http://dx.doi.org/10.1111/j.1600-0854.2009.00970.x.
21. Motley AM, Hettema EH. Yeast peroxisomes multiply by growth and division. J Cell Biol 2007; 178:399-410; PMID:17646399; http://dx.doi.org/10.1083/jcb.200702167.
22. Clauset A, Shalizi CR, Newman MEJ. Power-Law Distributions in Empirical Data. SIAM Rev 2009; 51:661-703; http://dx.doi.org/10.1137/070710111.
23. James A, Plank MJ, Edwards AM. Assessing Lévy walks as models of animal foraging. J R Soc Interface 2011; 8:1233-47; PMID:21632609; http://dx.doi.org/10.1098/rsif.2011.0200.
24. Newman MEJ. Power laws, Pareto distributions and Zipf's law. Contemp Phys 2005; 46:323-51; http://dx.doi.org/10.1080/00107510500052444.
25. Rhodes CJ, Anderson RM. Power laws governing epidemics in isolated populations. Nature 1996; 381:600-2; PMID:8637594; http://dx.doi.org/10.1038/381600a0.
26. Sornette D. Critical Phenomena in Natural Sciences, Chaos, Fractals, Self-organization and Disorder: Concepts and Tools. Springer, Berlin, 2006.
27. Viswanathan GM, Bartumeus F, Buldyrev SV, Catalan J, Fulco UL, Havlin S, et al. Levy flight random searches in biological phenomena. Physica A 2002; 314:208-13; http://dx.doi.org/10.1016/S0378-4371(02)01157-3.

28. Rapp S, Saffrich R, Anton M, Jäkle U, Ansorge W, Gorgas K, et al. Microtubule-based peroxisome movement. J Cell Sci 1996; 109:837-49; PMID:8718675.
29. Wiemer EA, Wenzel T, Deerinck TJ, Ellisman MH, Subramani S. Visualization of the peroxisomal compartment in living mammalian cells: dynamic behavior and association with microtubules. J Cell Biol 1997; 136:71-80; PMID:9008704; http://dx.doi.org/10.1083/jcb.136.1.71.
30. Heinemann P, Just WW. Peroxisomal protein import. In vivo evidence for a novel translocation competent compartment. FEBS Lett 1992; 300:179-82; PMID:1563518; http://dx.doi.org/10.1016/0014-5793(92)80191-I.
31. Islinger M, Cardoso MJ, Schrader M. Be different--the diversity of peroxisomes in the animal kingdom. Biochim Biophys Acta 2010; 1803:881-97; PMID:20347886; http://dx.doi.org/10.1016/j.bbamcr.2010.03.013.
32. Islinger M, Lüers GH, Li KW, Loos M, Völkl A. Rat liver peroxisomes after fibrate treatment. A survey using quantitative mass spectrometry. J Biol Chem 2007; 282:23055-69; PMID:17522052; http://dx.doi.org/10.1074/jbc.M610910200.
33. Lüers G, Hashimoto T, Fahimi HD, Völkl A. Biogenesis of peroxisomes: isolation and characterization of two distinct peroxisomal populations from normal and regenerating rat liver. J Cell Biol 1993; 121:1271-80; PMID:8509448; http://dx.doi.org/10.1083/jcb.121.6.1271.
34. Wiese S, Gronemeyer T, Ofman R, Kunze M, Grou CP, Almeida JA, et al. Proteomics characterization of mouse kidney peroxisomes by tandem mass spectrometry and protein correlation profiling. Mol Cell Proteomics 2007; 6:2045-57; PMID:17768142; http://dx.doi.org/10.1074/mcp.M700169-MCP200.
35. Angermüller S, Fahimi HD. Heterogenous staining of D-amino acid oxidase in peroxisomes of rat liver and kidney. A light and electron microscopic study. Histochemistry 1988; 88:277-85; PMID:2896644.
36. Delille HK, Agricola B, Guimaraes SC, Borta H, Lüers GH, Fransen M, et al. Pex11pbeta-mediated growth and division of mammalian peroxisomes follows a maturation pathway. J Cell Sci 2010; 123:2750-62; PMID:20647371; http://dx.doi.org/10.1242/jcs.062109.
37. Bonekamp NA, Sampaio P, de Abreu FV, Lüers GH, Schrader M. Transient complex interactions of mammalian peroxisomes without exchange of matrix or membrane marker proteins. Traffic 2012; 13:960-78.