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Organization and Transport of Peroxisomes in Neurons

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Role of Peroxisomes in Neurons

p0005 The potential role of peroxisomes in neurons is not well understood, except for the metabolism of very long-chain fatty acids. In human patients and in mice with peroxisome biogenesis disorders, neuronal migration is clearly affected. It is unclear whether this migration defect requires the function of peroxisomes in neuronal tissue or whether peroxisomes in nonneuronal tissues can provide this function. Experiments in mice show that the tissue-specific restoration of peroxisomes in the liver of mice deficient in peroxisome biogenesis does partially correct the neuronal migration deficits in the brain, but the restoration of peroxisomes in both tissues, rather than in only one, is more effective in restoring neuronal migration. It is also clear that a β-oxidation deficiency alone cannot cause neuronal migration defects, so other metabolic functions of peroxisomes may be involved. Mice lacking Pex11β, which is involved in peroxisome division, have only mild to no metabolic defects with respect to fatty acid β-oxidation and plasmalogen synthesis, but they still exhibit neuronal migration defects. These results emphasize that we still do not understand how or why peroxisomes affect neuronal migration and whether this is a direct or indirect effect.

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p0010 Peroxisomes are present in neural and astrocytic processes. The presence of peroxisomes in active growth cones with well-developed filopodia, varicosities, and neural terminal-like (presynaptic axon terminal) structures suggests their importance in the development and/or maintenance of neurites. Whereas many peroxisomes are found in long neurites with growth cones, only a small number exist in short neurites, leading to the suggestion that the short neurites are probably at rest since no growth cones have been observed. Therefore, it was postulated that the presence of peroxisomes in neurites helps their extending activity.

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Peroxisome Movement in Mammalian Cells

p0015 Based on their motility, peroxisomes fall into one of two groups. The first, comprising a majority (90–95%) of the peroxisomes in African green monkey kidney (CV1), Chinese hamster ovary (CHO), HepG2 cells, and neurites, exhibits randomly oriented, low-velocity movements (average velocity of 0.024 μm/s) without net organelle translocation in a distinct direction. This movement is independent of actin filaments and microtubules.

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The second category of peroxisomes, representing 5–10% of these organelles, exhibits fast movement (with average velocities of 0.26 μm/s, peak velocities of up to 0.75 μm/s, and sustained directional velocities up to 0.45 μm/s over distances of 11.5 μm), which is saltatory (discontinuous and erratic) and bidirectional (toward and away from the cell center) and results in the displacement of the organelle over distances of 10 μm. The bidirectional movement suggests the involvement of motor proteins of the dynein and/or kinesin families. Peroxisomes belonging to these two populations can spontaneously interchange and behave like the other. The fast movement, but not the slow kind, depends on energy in the form of ATP hydrolysis. Apart from these two movements, peroxisomes can also exist in a motionless 'frozen' state upon complete depletion of metabolic energy.

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Whereas the slow movement of peroxisomes is not affected by actin-depolymerizing agents, such as cytochalasins or anticytoskeletal drugs, microtubule-destabilizing agents, such as nocodazole, vinblastine, and demecolcine, completely abolish the fast, saltatory movement indicative of the involvement of microtubules in this process. Peroxisomes are often found in close proximity with microtubules when observed by triple confocal imaging of CV1 cells expressing GFP in peroxisomes, propidium iodide staining of the nucleus, and the immunolabeling of microtubules using anti-β-tubulin antibodies (**Figure 1**). In contrast to the microtubule-based movement of peroxisomes in mammalian cells, the movement of this organelle in fungi and plants depends on actin and myosin.

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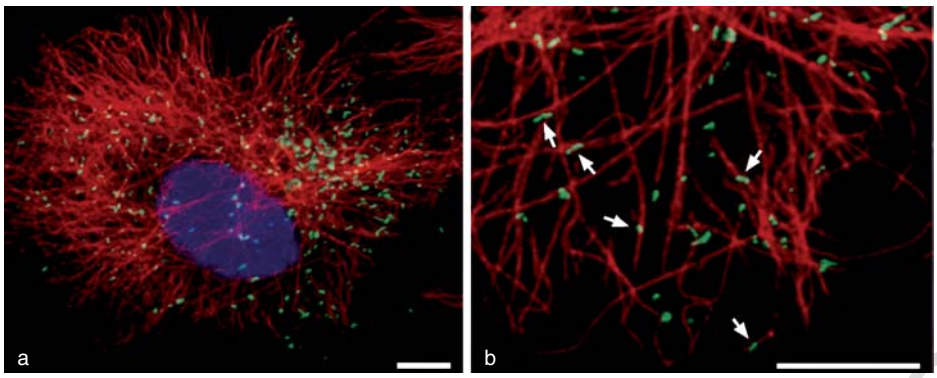
Peroxisome–Microtubule Binding *in Vitro*

p0030 Although the *in vivo* imaging of peroxisomes and microtubules has exhibited a close association between the two, formal evidence of peroxisome binding to microtubules has come from *in vitro* binding studies. A novel *in vitro* assay was developed in which purified rat liver peroxisomes associate with microtubules coated onto a microtiter plate (**Figure 2**). The binding was visualized using a confocal laser-scanning microscope and quantified by immunoblotting. The binding was time, temperature, and pH dependent.



f0005 **Figure 1** Association of peroxisomes with microtubules. Triple fluorescence confocal imaging of CV1 cells expressing GFP in peroxisomes (green), propidium iodide staining of the nucleus (pseudo-colored blue), and immunolabeling of microtubules using mouse anti-tubulin followed by goat anti-mouse IgG-CY5 (pseudo-colored red). (a) A single cell showing the overall distribution of peroxisomes relative to microtubules. (b) An enlarged portion of the image of the same cell showing the close association of many individual peroxisomes (arrows) with microtubules. Reprinted from Wiemer EA, Wenzel T, Deerinck TJ, Ellisman MH, and Subramani S (1997) Visualization of the peroxisomal compartment in living mammalian cells: Dynamic behavior and association with microtubules. *Journal of Cell Biology* 136: 71–80. Au11 Au12

p0035 Further investigation into this association showed that addition of ATP increased binding, whereas ATP depletion reduced binding. Stripping the peroxisome surface of peripheral membrane proteins by treatment with KCl reduced the binding, which was restored by the cytosol. However, treatment of either the peroxisomes or the cytosol with proteases or *N*-ethylmaleimide decreased binding and this was irreversible. These experiments clearly show the involvement of a peroxisomal membrane protein, probably a peroxisomal cytoplasmic linker protein (CLIP) and some cytosolic factor(s) for binding of peroxisomes to microtubules (**Figure 3**). When a motor-enriched fraction from chick embryo brain was added to the peroxisomes bound to microtubules, movement of the organelle occurred in an ATP-dependent manner, suggesting the involvement of microtubule-dependent motor proteins in peroxisome movement.

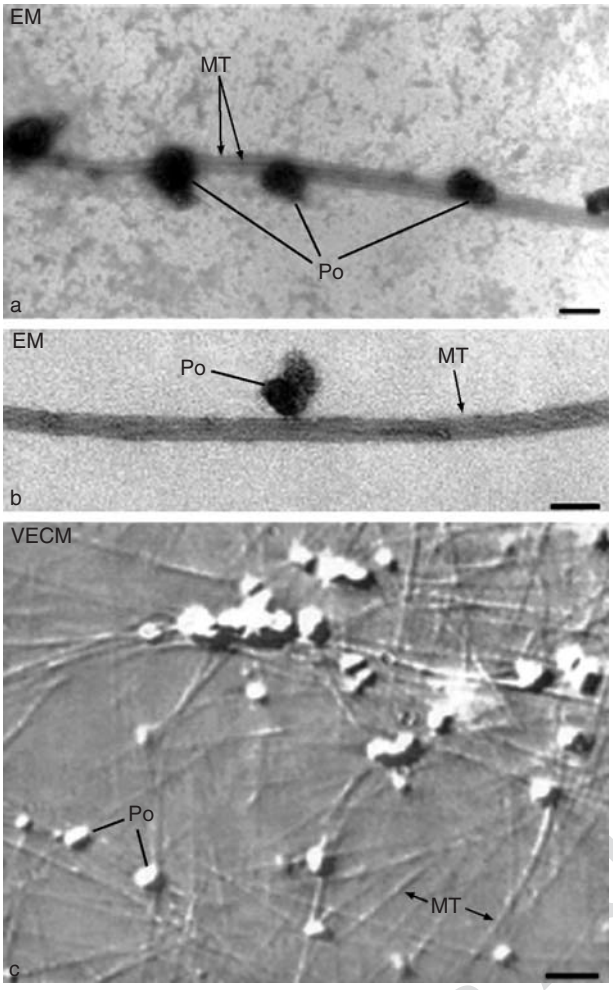
s0020 Motors Involved in Peroxisome Movement

p0040 Microtubule-dependent motor proteins, namely conventional kinesin (kinesin-1) and cytoplasmic dynein, are implicated in organelle trafficking and cell division. The use of RNAi to selectively inhibit several kinesins (kinesin-1, two members of the kinesin-2 family, three members of the kinesin-3 family, and a member of kinesin-14 or the C-terminal kinesin family) and cytoplasmic dynein in *Drosophila* S2 cells showed that only kinesin-1 and dynein heavy-chain motors are responsible for the movement of peroxisomes. The plus (+)-end directed kinesins carry organelles to the cell periphery, whereas the minus (--)-end directed dyneins ferry organelles back toward the center of the cell. The movement of peroxisomes in neurites of a subline of PC12 pheochromocytoma (PC12D) cells and *Drosophila* S2 cells was studied by time-lapse high-resolution fluorescence microscopy and by fluorescence imaging with one nanometer accuracy (FIONA), respectively, and found to occur in both the anterograde (cell center to cell periphery) and retrograde (cell periphery to cell center) directions (**Figure 4**). Overexpression of the dynamitin subunit of the dynein activator, dynactin, inhibited peroxisome movement in monkey kidney (COS7) cells. The tau protein, a neuronal microtubule-associated protein, inhibits the kinesin-dependent movement of peroxisomes in primary cortical neurons, retinal ganglion cells, and neuroblastoma cells and makes these cells more susceptible to oxidative stress.

p0045 Given the bidirectional movement of peroxisomes, the important question regarding whether the kinesin and dynein motors act simultaneously or sequentially on peroxisomes was addressed experimentally in *Drosophila* S2 cells. The conclusion from these studies was that the two kinds of motors did not act against each other simultaneously but, rather, up to 10 or 11 molecules of one or the other type of motor work together to produce up to 10 times the *in vitro* speed of peroxisome movement. These studies explain why the movement of peroxisomes is saltatory, fast, and occurs over distances that are far greater than the average step size of a single motor molecule.

Regulation of Peroxisome Motility s0025

p0050 Peroxisomal motility is sensitive to the extracellular environment and probably to receptor-mediated regulation in CHO and human umbilical vein endothelium cells. For example, the combination of ATP-S Au4



f0010 **Figure 2** Electron micrographs of peroxisomes bound to microtubules *in vitro*. Highly purified rat liver peroxisomes (Po) are specifically associating with taxol-stabilized microtubules from bovine brain (MT). (a and b) Electron micrographs (EM) of negatively stained suspended peroxisome–microtubule complexes with 2% phosphotungstic acid. (c) Video-enhanced contrast microscopy (VECM) of microtubules and peroxisomes. Scale bars = 0.2 μm (a), 0.05 μm (b), and 1 μm (c). Reprinted from Schrader M, Thiemann M, and Fahimi HD (2003) Peroxisomal motility and interaction with microtubules. *Microscopy Research and Technique* 61: 171–178.

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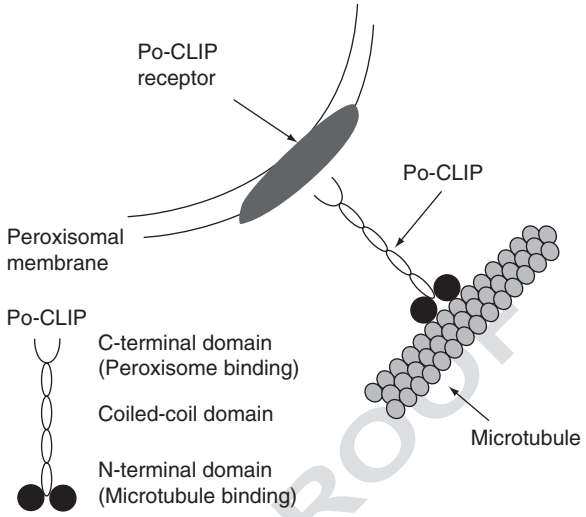

f0015 **Figure 3** Schematic model for the binding of peroxisomes to microtubules. Possible structure of the putative peroxisomal cytoplasmic linker protein (Po-CLIP) according to the model proposed for CLIP-170 and the presumed association of Po-CLIP to microtubules and peroxisomes. Reprinted from Thiemann M, Schrader M, Volkl A, Baumgart E, and Fahimi HD (2000) Interaction of peroxisomes with microtubules. *In vitro* studies using a novel peroxisome microtubule binding assay. *European Journal of Biochemistry* 267: 6264–6275.

Au14

and lysophosphatidic acid (LPA) blocks peroxisome movement probably by action via G-protein-coupled ATP and LPA receptors.

p0055 Peroxisome motility in CHO cells is regulated by a Au5 GTP-binding protein. Upon microinjection of GTP-S, a nonhydrolyzable GTP analog, there is a complete loss of peroxisome motility, although the effect is not immediate. A similar effect is elicited by activators of heterotrimeric G-proteins, such as aluminum fluoride and mastoparan.

p0060 Pertussis toxin, which antagonizes the action of the Au6 G_i/G_o class of proteins, prevents the action of GTP-S in inhibiting peroxisome motility, suggesting that this class of G-proteins is involved in the regulation of peroxisome movement. Additionally, inhibition of phospholipase C, classical protein kinase C isoforms, mitogen-activated kinase kinase, or phospholipase A2 (PLA2) affects the ability of this signaling pathway to inhibit peroxisome motility, so these could be signaling molecules acting downstream of the G-proteins. The PLA2 signaling cascade is believed to act via arachidonic acid, whose release inhibits peroxisome movement.

p0065 Although much of this information on the regulation of peroxisome movement comes from non-neuronal cells, the principles are likely to be conserved because microtubule-based peroxisome movement is common to mammals and the signaling cascades controlling peroxisome movement are widely distributed in mammalian tissues.

Peroxisome Morphology

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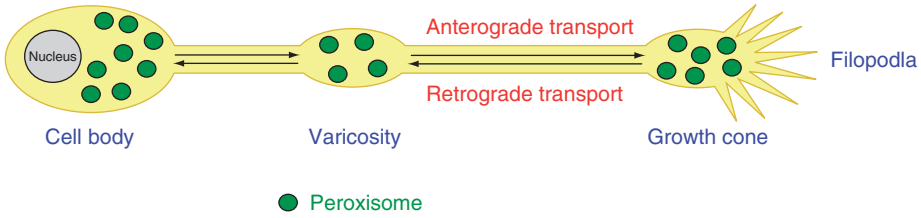
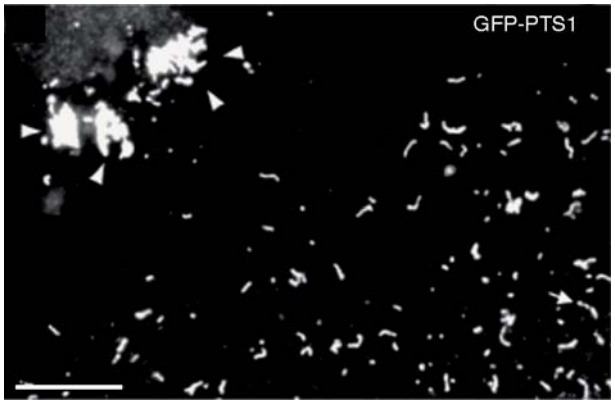
p0070 Although peroxisomes appear spherical in cross-section under the microscope, they are a highly dynamic subcellular compartment. Peroxisomes often exist in the form of an interconnected network or reticulum. They assume morphologically distinct shapes, including tubular structures that fuse to form a reticulum (**Figure 5**), when cultured cells are treated with defined growth factors (e.g., nerve growth



f0020 **Figure 4** A model of peroxisome transport from cell body to growth cone. Peroxisomes, which are formed in the cell body, move to the growth cone via the varicosity by anterograde transport. Peroxisomes play an important role at the growth cone and then return to the cell body by retrograde transport. During transportation, peroxisomes exhibit saltatory movement and/or are oscillating repeatedly. Reprinted from Ishikawa T, Kawai C, Sano M, and Minatogawa Y (2001) Peroxisomes exist in growth cones and move anterogradely and retrogradely in neurites of PC12D cells. *Experimental Cell Research* 266: 260–269. Au15



f0025 **Figure 5** GFP-PTS1 is targeted to peroxisomal aggregates in COS-7 cells, which contain elongated peroxisomes forming reticular structures. In some COS-7 cells, tubular peroxisomes and/or large peroxisomal aggregates (arrowheads) can be visualized by GFP-PTS1. An elongated peroxisome with a segmented appearance is visible (arrow). Reprinted from Schrader M, King SJ, Stroh TA, and Schroer TA (2000) Real time imaging reveals a peroxisomal reticulum in living cells. *Journal of Cell Science* 113: 3663–3671. Au16 Au17

factor) or with polyunsaturated fatty acids, free radicals, or ultraviolet (UV) irradiation. Even tissues engaged in the synthesis of special kinds of lipids exhibit this behavior. Both spherical and tubular peroxisomes move inside cells with similar velocities. Elongated peroxisomes could change shape while moving by shortening in length, collapsing into round structures, or elongating, and they also interacted with other peroxisomes.

p0075 Tubular peroxisomes of up to 5 μm can be induced in 40–50% of HepG2 cells by UV irradiation, and this induction occurs via the tyrosine kinase signal transduction pathway because it is sensitive to K 252b, an inhibitor of tyrosine kinase. Hydrogen peroxide also induces tubulation, but hypoxia reduces tubule formation. The overexpression of Pex11β results in tubulation and fission of elongated peroxisomes. Au7

p0080 The exact function and the precise mechanism of formation of these morphologically distinct peroxisomal compartments are still under investigation, but tubulation and fission of peroxisomes have been suggested to contribute to peroxisome proliferation and to the response of cells to reactive oxygen species.

Failure of Peroxisome Biogenesis and/or Movement Is Observed in Disease

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p0085 Whereas peroxisomes are abundant in normal hepatocytes and fibroblasts, their numbers are drastically decreased in peroxisome biogenesis disorders. These include the cerebrohepatorenal or Zellweger syndrome, rhizomelic chondrodysplasia punctata, and neonatal adrenoleukodystrophy. Although diseased cells lack detectable peroxisomes when analyzed using antibodies to peroxisomal matrix markers, remnant membranous structures termed 'ghosts' with peroxisomal membrane proteins (PMPs) are present. The presence of these ghosts with PMPs is explained by the fact that the signals and machineries involved in peroxisomal matrix and membrane protein import are different, and most (but not all) of these biogenesis disorders affect only matrix protein import.

p0090 Peroxisomes are assumed to play a crucial role in the formation of cortex, cerebellum, and the inferior olivary nucleus because there are severe morphogenic defects in these tissues in peroxisome biogenesis disorders. Analysis of peroxisome-deficient fetuses with focus on neurodevelopment showed defects typical of Zellweger syndrome, such as a defect in the neuronal migration in the neocortex, delay in neuronal maturation, increased apoptosis in the cortical plate, and structural abnormalities in the inferior olivary nucleus.

p0095 Human patients lacking the multifunctional protein 2 (MFP2), a D-bifunctional protein with enoyl-CoA hydratase and 3-hydroxy-acyl-CoA dehydrogenase Au8

activities, necessary for peroxisomal β-oxidation of bile acid intermediates and pristanic acid, were found to have neuronal migration defects. MFP2 is involved in the metabolism of very long-chain fatty acids (VLCFAs). The accumulation of VLCFAs as a result of peroxisomal *β*-oxidation deficiency, depletion of plasmalogens, or reduced content of the polyunsaturated fatty acid docosahexaenoic acid (DHA) play a major role in impairment of neuronal migration. However, in mice lacking MFP2, the neuronal migration defect was not obvious.

p0100 Au9 In fibroblasts from human patients with Pex1 or D-bifunctional enzyme deficiencies, peroxisome abundance, movement, and association with microtubules are affected. This is also true in mice lacking Pex13. Perhaps these are deficient in the recruitment of a protein that links peroxisomes with microtubules.

s0040 Peroxisome Biogenesis Disorders That Affect Neuronal Migration

p0105 Peroxisome biogenesis disorders result from defects in *PEX* genes, which code for proteins involved in peroxisome biogenesis and protein import. An important pathological feature of peroxisome biogenesis disorders is defective neuronal migration.

p0110 The following are peroxisome biogenesis disorders:

1. Zellweger syndrome or cerebro-hepato-renal syndrome results in abnormal central nervous system neuronal migration due to the lack of functional peroxisomes. Zellweger syndrome phenotype is caused by mutations in any of several *PEX* genes, such as *PEX1*, *PEX2*, *PEX3*, *PEX5*, *PEX6*, *PEX10*, *PEX12*, *PEX13*, *PEX16*, *PEX19*, or *PEX26*. The most striking pathological abnormalities are apparent in the brain. As indicated previously, most of these affect peroxisomal matrix protein import, but a few, such as *PEX3*, *PEX16*, and *PEX19*, affect both PMP and matrix protein import.
2. Neonatal adrenoleukodystrophy is caused by milder mutations in the genes affected in Zellweger syndrome.
3. Infantile Refsum disease (IRD) is the mildest form of impairment among peroxisomal biogenesis disorders. It is also called infantile phytanic acid storage disorder. The biochemical abnormalities are not restricted to phytanic acid but also include accumulation of VLCFAs, di- and trihydroxycholestanoic acid, and pipecolic acid. Mutations in *PEX1*, *PEX2*, or *PEX26* can cause IRD.
4. Rhizomelic chondrodysplasia punctata (RCDP): Biochemically, RCDP patients have subnormal levels of red cell plasmalogens and progressive accumulation of phytanic acid. Mutations in the PTS2 receptor, *PEX7*, cause RCDP.
5. Zellweger-like syndrome.

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See also: Organization and functions of Neuronal microtubules (00730); Organization and transport of lysosomes and endosomes in neurons (00733); Organization and transport of mitochondria and neurons (00734).

Further Reading

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