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The control of peroxisome number and size during division and proliferation

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Like other subcellular organelles, peroxisomes divide and segregate to daughter cells during cell division, but this organelle can also proliferate or be degraded in response to environmental cues. Although the mechanisms and genes involved in these processes are still under active investigation, an important player in peroxisome proliferation is a dynamin-related protein (DRP) that is recruited to the organelle membrane by a DRP receptor. Related DRPs also function in the division of mitochondria and chloroplasts. Many other proteins and signals regulate peroxisome division and proliferation, but their modes of action are still being studied.

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Introduction

Peroxisomes are ubiquitous organelles that are intimately involved in lipid metabolism [1]. Like other subcellular organelles, they divide and segregate either to distribute themselves within cells or to endow daughter cells with peroxisomes. Peroxisome segregation within or between cells requires organelle movement on either actin filaments (in fungi and plants) [2–5] or microtubule-based filaments (in mammals) [6–9]. This review focuses only briefly on peroxisome movement, a topic that has been discussed recently [1], and then outlines why and how peroxisome number and size are regulated. Where relevant, we draw upon parallel mechanisms used by other organelles, such as mitochondria and chloroplasts.

Cells stringently regulate organelle number, volume, size and content in response to environmental signals. Regulation of these properties, together with organelle movement, allows cells to respond to metabolic or environmental stress, cope with the needs of cell division or differentiation, remove excess or damaged organelles by turnover, correct imbalances in organelle segregation during cell division or repopulate organelles with different enzymes upon switching to a new environment. The division of some organelles, such as the nucleus or the Golgi apparatus, is coupled to the cell cycle and checkpoints are triggered by the delay or inhibition of organelle division [10]. However, for others, such as mitochondria and chloroplasts, division is uncoupled from cell division [11]. Peroxisomes can also divide separately from cell division.

In single-cell or multicellular eukaryotes, there are three major pathways for the control of peroxisome number. First, in constitutively dividing cells, peroxisomes divide, as mitochondria and chloroplasts do, by fission of preexisting peroxisomes, a process we refer to simply as 'peroxisome division' [12,13]. This process regulates peroxisome number incrementally in a geometric manner. A second general pathway is one by which peroxisomes are induced to create many new organelles within a short period of time, a process we call 'peroxisome proliferation', which increases organelle number much more rapidly than would be possible by sequential peroxisome division. Finally, peroxisome number is also controlled by 'peroxisome turnover', which involves autophagic processes [14].

In fungi and yeasts, peroxisome division occurs during constitutive growth and during cell division. Peroxisome size and number are sensitive to peroxisomal metabolic pathways and metabolites [15–17]. However, peroxisome number and size can also be induced by peroxisome proliferation, which generally occurs when cells are shifted to nutrients whose metabolism requires peroxisomes and their constituent enzymes [1,13]. Peroxisome turnover is manifested when yeasts are shifted from carbon sources whose metabolism requires peroxisomes to media whose utilization does not require this organelle. This process, termed pexophagy, has mechanistic similarities to autophagic processes involving degradation in the yeast vacuole (the equivalent of lysosomes) [14]. Peroxisome volume changes in response to the import of matrix proteins [13]. Finally, peroxisomal contents can also vary, as shown for methylotrophic yeasts, in which peroxisomes are populated with enzymes involved in fatty-acid β-oxidation during growth on oleate, but new peroxisomes are endowed with methanol-assimilation enzymes upon growth on methanol [13].

Peroxisome movement on cytoskeletal elements

Peroxisomes exhibit two kinds of movement: one is slow and non-directional and the other is rapid and directional [3,6,7].

Peroxisomes colocalize with actin microfilaments in yeasts and plants [2,5]. Disruption or stabilization of either microtubules or actin filaments with drugs reveals that the rapid movement of peroxisomes in these organisms requires actin filaments but not microtubules [2,5]. Directional movement of peroxisomes may be especially important during cell division in yeast when peroxisomes are transported from mother to daughter cell. Type V myosin is the motor, since its chemical inhibitor, butanedione monoxime, blocks the movement [3]. Myo2p (one of two yeast type-V myosins) localizes peroxisomes to the bud at early stages of the cell cycle [2].

In mammalian cells, peroxisomes associate with microtubules *in vivo* [6,7,18–20] and *in vitro* [18,20]. Peroxisome movement occurs randomly in all areas of the cytoplasm but its physiological role is unclear [6,7,20]. Dynein, kinesin and the dynactin complex act as motor molecules, as shown by *in vitro* observations of peroxisome movement and *in vivo* experiments using either antibody microinjection or overexpression of the negative regulator dynamitin [20]. However, the target for the cytoskeleton-based motors on peroxisomes remains unknown.

In yeast grown in oleate, the small GTPase Rho1p is recruited to peroxisomes via interaction with the peroxisome-associated protein Pex25p, although it also interacts with Pex30p [21•]. A *rho1(ts)* mutant which is non-functional at the restrictive temperature shows fewer and smaller peroxisomes and reduced cargo import efficiency, and mislocalizes actin patches to the peroxisomes. Actin patches are found on peroxisomes when *RHO1* is mutated, or when Rho1p is not recruited to peroxisomes (as seen in *pex25Δ* cells), suggesting that Rho1p is needed for the reorganization of actin on peroxisomes. Rho1p and actin reorganization might be necessary for the fusion of immature peroxisome precursors, as is known to be the case in *Yarrowia lipolytica* [22], but its precise role is still unclear.

Peroxisome division and dynamin-related proteins

Dynamin-related proteins (DRPs) are large GTPases involved in many processes including the division of mitochondria and chloroplasts [23••]. They are functionally related to the FtsZ proteins of bacteria, plants and primitive eukaryotes (Table 1) [11]. During bacterial cell division, FtsZ, a self-assembling GTPase, forms a ring on the cytoplasmic face of the cytoplasmic membrane, providing a scaffold for the recruitment of additional proteins to the cell center, and may also force membrane deformation and constriction. In plant chloroplasts, stroma-targeted FtsZ forms a ring on the inner membrane of chloroplasts to execute division. Primitive eukaryotes have mitochondrial FtsZ that similarly assembles into a ring in the matrix. However, mitochondria in fungi, animals and plants lack FtsZ, but use a DRP that facilitates mitochondrial division from the cytosolic side of mitochondrial membranes. Dnm1 in yeast and DLP1/Drp1 in animals associate with the cytosolic face of the mitochondrial outer membrane. *Arabidopsis* uses a DRP called ADL2 for mitochondrial division [24,25].

Peroxisome division also requires a DRP. In yeast, the *vps1* mutant exhibits only one or two giant peroxisomes

Table 1

Membrane fission components in bacteria, chloroplasts, mitochondria and peroxisomes.

		Bacteria	Chloroplasts	Mitochondria	Peroxisomes
Inside	Self-assembling GTPase related to bacterial FtsZ	FtsZ	FtsZ1 FtsZ2	FtsZ-mt (primitive eukaryotes only)	
	Positioning of the fission ring	MinD–MinC MinE	MinD MinE		
	Chaperone		ARC6		
	Assembly of the division apparatus		ARTEMIS (YidC/Oxa1/Alb3) – related		
	Lipid-reorganizing proteins			Aox–Pex16p (*Y. lipolytica*)	
Outside	Self-assembling GTPase related to dynamin		ARC5 (plant) Dnm2 (red alga)	Dnm1 (yeast) Adl2 (plant) DLP1/Drp1 (mammal)	Vps1p (yeast) DLP1/Drp1 (mammal) APM1/DRP3A (plant)
	DRP receptor			Fis1 (mammal/yeast)	Fis1 (mammal)
	Ring assembly or other function			Fis2p/Mdv1p (yeast) Erp-1 (worm) Endophilin B (mammal)	

that may form long tubules oriented along actin cables [2]. In mammalian cells, biochemical and *in vivo* visualization indicate that a subpopulation of DLP1/Drp1 colocalizes with peroxisomes. Expression of a dominant-negative DLP1(K38A) form, which is deficient in GTP hydrolysis, or knock-down of the protein level by siRNA-mediated silencing results in the reduction of peroxisome abundance and the formation of tubular peroxisomes. This effect is dramatically enhanced when combined with Pex11β overexpression, which increases peroxisome proliferation. Tubular membranes are observed to have constrictions, but are unable to divide, suggesting that the DRP acts at a later stage in the peroxisomal division process to pinch off small peroxisomes from constricted tubules [26,27,28••].

Although FtsZ and DRPs act either as mechanochemical transducers or as regulatory GTPases, they are not strictly equivalent to each other. The FtsZ ring, when used for organelle division, forms on the lumenal side of the organelle membranes, whereas DRPs act on the cytosolic side [11]. Furthermore, in organelles such as chloroplasts, that use the FtsZ ring in the stroma and a DRP ring on the cytosolic side, the DRP ring acts late in division, after the FtsZ ring has constricted the membrane from within the organelle. Even in endocytosis, dynamin acts at a late stage after clathrin and other coat proteins have constricted the neck of endocytic vesicles [11].

Even during peroxisome division, the DRP ring acts late, after some other unknown machinery has constricted the membranes [26,27,28••]. One example of a protein that could control membrane constriction from within the organelle is *Y. lipolytica* Pex16p, an intraperoxisomal peripheral membrane protein that negatively regulates division. Import of the matrix protein acyl-CoA oxidase into peroxisomes titrates the Pex16p and relieves the inhibition of peroxisome division [12]. The mechanism by which Pex16p remodels lipids in the peroxisomal membrane (described below) may be very different from the mechanism by which the FtsZ ring achieves membrane constriction.

As DRPs lack the dynamin pleckstrin homology (PH) domain that binds membrane lipids, some other factors must determine their association with peroxisomes. Fis1, a DLP1-interacting protein known to function in the mitochondrial fission, was recently found to function in peroxisome fission or fragmentation as well. Fis1 has a transmembrane domain and C-terminal tail that target it to the peroxisome membrane. Overexpression of Fis1 promotes peroxisome division while its silencing causes tubulation in mammalian cells [29•].

Proteins involved in peroxisome proliferation, their functions and interactions

The great diversity in the shape, size and number of peroxisomes has been actively investigated and has led to the discovery of several *PEX* genes (Table 2). *PEX* genes primarily function in the targeting and import of peroxisomal proteins but some are also involved in peroxisome division and proliferation. The roles of these proteins in

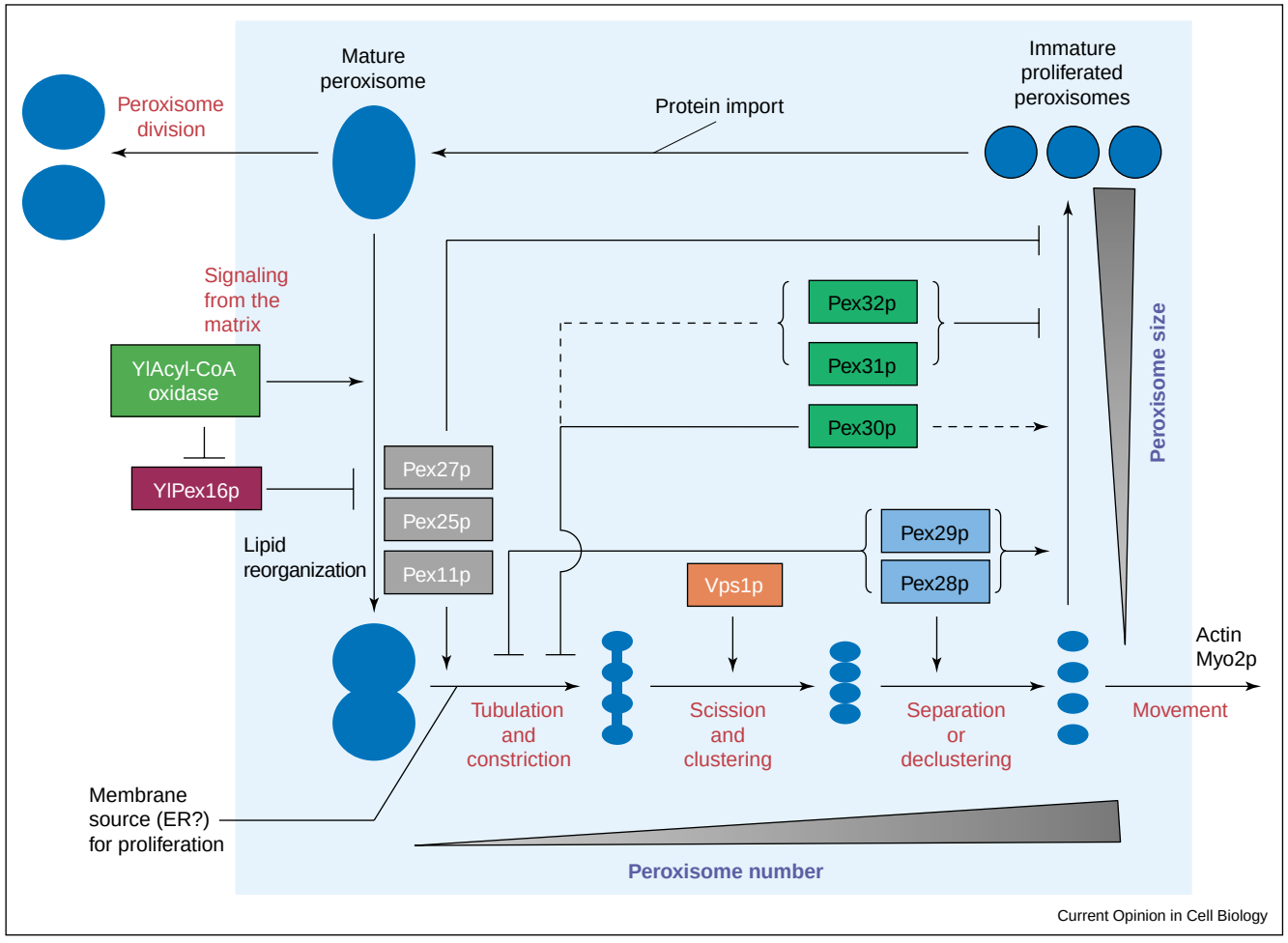
Table 2

PEX genes involved in the regulation of peroxisome size and number.

Gene	Reported in	Deletion phenotypes	Overexpression	References
PEX11	Sc, Tb, Rattus sp, Hs (α,β,γ)	Fewer and larger peroxisomes	Smaller and numerous peroxisomes in *pex11*Δ or *pex25*Δ cells	[30,34,47–49]
PEX16	Yl	Excessive proliferation of immature peroxisomal vesicles	Reduced number of greatly enlarged peroxisomes	[50]
PEX25 (YPL112C)	Sc	Fewer and enlarged peroxisomes	Restored normal peroxisomes in *pex25*Δ, *pex27*Δ, *pex28*Δ or *pex29*Δ strains; large, clustered peroxisomes in *pex11*Δ cells	[31–33,36]
PEX27 (YOR193W)	Sc	Fewer and enlarged peroxisomes	Restored normal peroxisomes in *pex27*Δ, smaller peroxisomes in *pex25*Δ and large, clustered organelles in *pex11*Δ cells	[32,33]
PEX28 (YHR150W)	Sc, Yl (*PEX24*)	More, but smaller and clustered	Restored normal peroxisome size and number in *pex28*Δ strain	[35,36]
PEX29 (YDR479C)	Sc, Yl (*PEX24*)	peroxisomes with thickened membrane between peroxisomes	Restored normal peroxisome morphology in *pex29*Δ strain	[36]
PEX30 (YLR324W)	Sc, Yl (*PEX23*)	Increased peroxisome number but size is normal	Restored normal peroxisome size and number in *pex30*Δ strain	[37,38•]
PEX31 (YGR004W)	Sc, Yl (*PEX23*)	Increased number and enlarged peroxisomes	Restored normal peroxisome size and number in *pex31*Δ strain; restored normal peroxisome size and number in cells lacking either Pex30p or Pex32p, or both	[38•]
PEX32 (YBR168W)	Sc, Yl (*PEX23*)	Increased number and enlarged peroxisomes	Restored normal peroxisome size and number in *pex32*Δ strain	[38•]

Hs, Homo sapiens; Sc, Saccharomyces cerevisiae; Tb, Trypanosoma brucei; Yl, Yarrowia lipolytica;

Figure 1



Working model for proteins involved in the control of peroxisome division, proliferation, number, size and movement. The model is based on information in yeast systems (*Y. lipolytica and S. cerevisiae*), in which many of the genes have been defined [2,12,17,21•,30–36,38•],47. Only mature peroxisomes undergo division. Events depicted within the blue box occur during peroxisome proliferation. Peroxisomes divide and segregate to daughter cells even when key proteins (e.g. Vps1p or Pex11p) involved in proliferation are missing. The membrane source for peroxisome proliferation has been suggested to be the endoplasmic reticulum. Peroxisome number increases from left to right and size increases from the bottom to the top of the figure. Although changes in peroxisome size and number are shown on different axes, they probably occur simultaneously as inter-related, rather than independent, events. With the exception of Pex16p and acyl-CoA oxidase, which are from *Y. lipolytica*, all other Pex proteins shown are from *S. cerevisiae*. Pex proteins shown in boxes of the same color are either related or redundant with each other. Single-headed arrows indicate positive regulators and arrows ending in perpendicular bars show negative regulators. Dotted lines indicate weak effects.

the control of peroxisome number and size in yeast are illustrated in Figure 1 and the interactions between them are displayed in Table 3.

PEX11, PEX25 and PEX27

While *S. cerevisiae PEX11* was cloned long ago [30], the newer members of this family were isolated more recently. *PEX25* was discovered as a gene that was induced in yeast cells upon growth on oleate [31]. *PEX27*, expressing a peroxisomal membrane protein constitutively at a low level, was found by homology to *PEX25* [32,33]. These *S. cerevisiae* genes in the *PEX11* family share significant sequence similarity in their C-terminal segments [32,33]. In addition, all members homo-oligomerize, a feature essential for their function [33]. In *S. cerevisiae*, Pex11p homo-oligomerization correlates with inhibition of function, and a point mutation disrupting this self-association causes hyper-proliferation of peroxisomes [34].

Either single or pairwise deletions of *PEX11*/*PEX25*/*PEX27* cause peroxisomes to be greatly enlarged and dense [32]. Deletion of *PEX11* alone, but not of *PEX25* or *PEX27*, also affected growth on oleate, as was also the case for all double-mutant combinations of these three genes. A triple mutant (*pex11Δ pex25Δ*

Table 3

Interactions among proteins affecting peroxisome number and size.

	Pex11p	Pex25p	Pex27p	Pex28p	Pex29p	Pex30p	Pex31p	Pex32p	Rho1p
Pex11p	++								
Pex25p		+							
Pex27p	+	++	+						
Pex28p									
Pex29p					++				
Pex30p					++	+			
Pex31p						++	++		
Pex32p				+		+			
Rho1p		++				++			

+ and ++ indicate weak and strong interactions, respectively.

pex27Δ) shows a severe peroxisomal protein import defect and is unable to utilize oleate for growth [33].

PEX28 and PEX29

Y. lipolytica cells grown in oleic acid showed increased levels of YlPex24p. A *pex24Δ* strain was defective in the targeting of matrix and membrane proteins but contained some peroxisomal proteins [35]. *S. cerevisiae* has two homologs, *PEX28* and *PEX29*, of *YlPEX23*. Single or double deletions of *PEX28/PEX29* result in increased numbers of smaller peroxisomes that cluster together, suggesting that these proteins are involved in the separation of peroxisomes [36]. The same study also noted that overexpression of *PEX25* or *VPS1*, but not *PEX11*, restored the wild-type phenotype to the cells deleted for either *PEX28* or *PEX29* or both these genes.

PEX30, PEX31 and PEX32

In *Y. lipolytica*, deletion of *PEX23* resulted in the absence of mature peroxisomes, but small vesicular structures containing matrix and membrane proteins were observed [37]. *S. cerevisiae* has three homologues — *PEX30*, *PEX31* and *PEX32* — of *YlPEX23*. Deletion of *PEX30* leads to increased numbers of peroxisomes, while deletion of *PEX31* and *PEX32* results in enlarged peroxisomes. Deletion of all three genes causes a tremendous increase (fivefold) in the average number of peroxisomes per cell, suggesting that Pex30p negatively regulates peroxisome number whereas Pex31p and Pex32p negatively control peroxisome size. These proteins are not redundant in their function, as neither Pex30p nor Pex32p can functionally substitute for one another or for Pex31p. *PEX28* and *PEX29* function upstream of *PEX30*, *PEX31* and *PEX32* [38•].

It remains to be seen whether the pathways of peroxisome division and proliferation are only variations on a common theme. Most of the genes involved in peroxisome fission (e.g. Pex27p, Pex28p, Pex29p and Pex31p) are not induced by incubation of cells in oleic acid. Whether a cell is in an induced or a non-induced state, it is possible that the same genes control peroxisome division and proliferation. Perhaps it is the rate of division of peroxisomes that increases in the induced state.

Signals and events leading to peroxisome proliferation

In *Y. lipolytica*, peroxisome biogenesis under induction conditions proceeds via the generation of a series of five vesicular intermediates (called P1–P5), with defined densities and protein/lipid compositions, before mature peroxisomes (P6 compartment) appear [22]. The process depends on a metabolic signal emanating from the peroxisome lumen [12] (Fig. 1). The regulated interaction between Pex16p and a hetero-pentameric complex of acyl-CoA oxidase (Aox) plays a crucial role in the mechanism of proliferation. Pex16p, which is present on the matrix side of the peroxisomal membrane, prevents the division and excessive proliferation of the immature peroxisomal vesicles that are still growing in size by importing proteins and lipids. The hetero-pentameric complex of Aox, which is present in the matrix of premature peroxisomes, is redistributed to the peroxisomal membrane only in mature peroxisomes. This, in turn leads to an interaction between the membrane-bound Aox complex and Pex16p, forming a supramolecular complex containing two molecules of each. The complex thus formed eliminates the negative action of Pex16p on the scission of peroxisomal membrane, enabling the division of mature peroxisomes.

How does the interaction between Pex16p and Aox promote membrane curvature and fission? Changes in the local lipid composition in the inner and outer leaflets of the lipid bilayer at the site of peroxisome membrane constriction are proposed to play a role in peroxisome division [39] (V Titorenko, personal communication). The increase in levels of the cone-shaped lipids phosphatidic acid (PA) and diacylglycerol (DAG) in the outer leaflet, may cause the membrane curvature. Peroxisome division coincides with a reduction in the concentration of LPA and an increase in the PA and DAG concentration only in the P6 peroxisomes, and not in any of its precursors (P1–P5). This would explain why only mature

peroxisomes, and not their precursors, divide. LPA is converted to PA and then to DAG via the action of an LPA acyltransferase followed by a phosphatase. The high concentration of LPA is proposed to be maintained in the inner leaflet of the peroxisomal membrane by the inhibition of LPA acyltransferase activity by Pex16p. When this inhibition is relieved by the interaction of Pex16p with Aox, LPA is converted to DAG (via PA), and the DAG spontaneously flips to the outer leaflet, resulting in the membrane curvature necessary for peroxisome division [39] (V Titorenko, personal communication).

By contrast, in human cells peroxisome proliferation may not depend on peroxisomal metabolism, because *pex* mutants deficient in matrix protein import do proliferate and segregate peroxisome remnants to daughter cells [1]. Instead, in mammalian cells, signals mimicked by peroxisome proliferators activate the transcription factors PPARα and PPARγ, which induce the transcription of PEX11α [40]. Not surprisingly, the overexpression of Pex11α or Pex11β alone, in the absence of other signals or peroxisomal metabolism, is sufficient to cause peroxisome proliferation in mammalian cells [41,42]. Mammalian PEX11 indirectly promotes peroxisome proliferation by recruiting DLP1/Drp1 to the peroxisome membrane [26]. Although the natural signals causing peroxisome proliferation are not fully characterized, in HepG2 cells specific growth factors and polyunsaturated fatty acids, such as arachidonic acid, mediate peroxisome tubulation and proliferation [43]. Additionally, in mammalian cells, proliferation of tubular peroxisomes is stimulated by exposure of the cells to UV radiation or to reactive oxygen species [44]. Thus, in contrast to *Y. lipolytica*, where signals for division emanate from within peroxisomes, proliferation in mammalian cells appears to be initiated by extracellular signals. These activate intracellular signaling cascades, leading to a transcriptional response culminating in the increased expression of proteins that mediate peroxisome tubulation, constriction and scission to produce many peroxisomes.

Similarly, in *S. cerevisiae* the transcription factors Pip2p/Oaf1p [45] and Adr1p [46] are activated when the cells are switched to oleate medium. Both induce expression of Pex11p, and Pip2p/Oaf1p activates Pex25p expression to cause peroxisome proliferation while also elevating proteins involved in fatty-acid oxidation.

Conclusions

Progress in recent years has revealed that peroxisomes, like plant chloroplasts and mitochondria of fungal, plant and animal origin, divide using DRPs. The surprise, however, is that many genes affecting peroxisome biogenesis also have an impact on peroxisome size and number. Proteins encoded by some of these genes modulate peroxisomal membrane lipids from within the organelle. However, much remains to be discovered regarding the recruitment and regulation of peroxisome-associated DRPs, the mechanism of constriction of tubular peroxisomes before the action of DRPs, the molecular motors that couple peroxisomes to the cytoskeleton during peroxisome segregation, the mechanism by which the division site on the membrane is selected, and the signaling events that coordinate peroxisome biogenesis with division and proliferation.

Update

A new method called fluorescence imaging with one nanometer accuracy (FIONA) shows that multiple kinesins or multiple dyneins work together *in vivo* during peroxisome movement, producing up to 10 times the *in vitro* speed for peroxisome movement [51].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lazarow PB: **Peroxisome biogenesis: advances and conundrums**. *Curr Opin Cell Biol* 2003, **15**:489-497.

2. Hoepfner D, van den Berg M, Philippsen P, Tabak HF, Hettema EH: **A role for Vps1p, actin, and the Myo2p motor in peroxisome abundance and inheritance in *Saccharomyces cerevisiae***. *J Cell Biol* 2001, **155**:979-990.

3. Jedd G, Chua NH: **Visualization of peroxisomes in living plant cells reveals acto-myosin-dependent cytoplasmic streaming and peroxisome budding**. *Plant Cell Physiol* 2002, **43**:384-392.

4. Mano S, Nakamori C, Hayashi M, Kato A, Kondo M, Nishimura M: **Distribution and characterization of peroxisomes in *Arabidopsis* by visualization with GFP: dynamic morphology and actin-dependent movement**. *Plant Cell Physiol* 2002, **43**:331-341.

5. Mathur J, Mathur N, Hulskamp M: **Simultaneous visualization of peroxisomes and cytoskeletal elements reveals actin- and not microtubule-based peroxisome motility in plants**. *Plant Physiol* 2002, **128**:1031-1045.

6. Rapp S, Saffrich R, Anton M, Jakle U, Ansorge W, Gorgas K, Just WW: **Microtubule-based peroxisome movement**. *J Cell Sci* 1996, **109**:837-849.

7. 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.

8. Thiemann M, Schrader M, Volkl A, Baumgart E, Fahimi HD: **Interaction of peroxisomes with microtubules: *in vitro* studies using a novel peroxisome-microtubule binding assay**. *Eur J Biochem* 2000, **267**:6264-6275.

9. Schrader M, Thiemann M, Fahimi HD: **Peroxisomal motility and interaction with microtubules**. *Microsc Res Tech* 2003, **61**:171-178.

10. Sutterlin C, Hsu P, Mallabiabarrena A, Malhotra V: **Fragmentation and dispersal of the pericentriolar Golgi complex is required for entry into mitosis in mammalian cells**. *Cell* 2002, **109**:359-369.

11. Osteryoung KW, Nunnari J: **The division of endosymbiotic organelles**. *Science* 2003, **302**:1698-1704.

12. Guo T, Kit YY, Nicaud JM, Le Dall MT, Sears SK, Vali H, Chan H, Rachubinski RA, Titorenko VI: **Peroxisome division in the yeast *Yarrowia lipolytica* is regulated by a signal from inside the peroxisome**. *J Cell Biol* 2003, **162**:1255-1266.

13. Veenhuis M, Kiel JA, Van Der Klei IJ: **Peroxisome assembly in yeast**. *Microsc Res Tech* 2003, **61**:139-150.

14. Farré JC, Subramani S: **Peroxisome turnover by micropexophagy: an autophagy-related process**. *Trends Cell Biol* 2004, **14**:515-523.

15. Poll-The BT, Roels F, Ogier H, Scotto J, Vamecq J, Schutgens RB, Wanders RJ, van Roermund CW, van Wijland MJ, Schram AW *et al.*: **A new peroxisomal disorder with enlarged peroxisomes and a specific deficiency of acyl-CoA oxidase (pseudo-neonatal adrenoleukodystrophy)**. *Am J Hum Genet* 1988, **42**:422-434.

16. Chang CC, South S, Warren D, Jones J, Moser AB, Moser HW, Gould SJ: **Metabolic control of peroxisome abundance**. *J Cell Sci* 1999, **112**:1579-1590.

17. Smith JJ, Brown TW, Eitzen GA, Rachubinski RA: **Regulation of peroxisome size and number by fatty acid β-oxidation in the yeast *Yarrowia lipolytica***. *J Biol Chem* 2000, **275**:20168-20178.

18. Schrader M, Burkhardt JK, Baumgart E, Luers G, Spring H, Volkl A, Fahimi HD: **Interaction of microtubules with peroxisomes: tubular and spherical peroxisomes in HepG2 cells and their alterations induced by microtubule-active drugs**. *Eur J Cell Biol* 1996, **69**:24-35.

19. Huber CM, Saffrich R, Ansorge W, Just WW: **Receptor-mediated regulation of peroxisomal motility in CHO and endothelial cells**. *EMBO J* 1999, **18**:5476-5485.

20. Schrader M, King SJ, Stroh TA, Schroer TA: **Real time imaging reveals a peroxisomal reticulum in living cells**. *J Cell Sci* 2000, **113**:3663-3671.

21. • Marelli M, Smith JJ, Jung S, Yi E, Nesvizhskii AI, Christmas RH, Saleem RA, Tam YY, Fagarasanu A, Goodlett DR *et al.*: **Quantitative mass spectrometry reveals a role for the GTPase Rho1p in actin organization on the peroxisome membrane**. *J Cell Biol* 2004, **167**:1099-1112.
The first evidence showing that a subpopulation of Rho1p is associated with oleate-induced peroxisomes through its interaction with peroxisomal membrane protein Pex25p, and affects actin reorganization on peroxisomes. Rho1p function is not yet fully understood.

22. Titorenko VI, Rachubinski RA: **The life cycle of the peroxisome**. *Nat Rev Mol Cell Biol* 2001, **2**:357-368.

23. •• Praefcke GJ, McMahon HT: **The dynamin superfamily: universal membrane tubulation and fission molecules?** *Nat Rev Mol Cell Biol* 2004, **5**:133-147.
An excellent review of the role of DRPs in membrane tubulation and fission.

24. Arimura S, Tsutsumi N: **A dynamin-like protein (ADL2b), rather than FtsZ, is involved in *Arabidopsis* mitochondrial division**. *Proc Natl Acad Sci USA* 2002, **99**:5727-5731.

25. Arimura S, Aida GP, Fujimoto M, Nakazono M, Tsutsumi N: ***Arabidopsis* dynamin-like protein 2a (ADL2a), like ADL2b, is involved in plant mitochondrial division**. *Plant Cell Physiol* 2004, **45**:236-242.

26. Li X, Gould SJ: **The dynamin-like GTPase DLP1 is essential for peroxisome division and is recruited to peroxisomes in part by PEX11**. *J Biol Chem* 2003, **278**:17012-17020.

27. 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**:8597-8605.

28. •• Koch A, Schneider G, Luers GH, Schrader M: **Peroxisome elongation and constriction but not fission can occur independently of dynamin-like protein 1**. *J Cell Sci* 2004, **117**:3995-4006.
Fluorescence and electron microscopic observation of DLP1-silenced cells clearly shows that DLP1 functions at a late stage of fission during peroxisome division. Similar effects are also found in other organelles such as the mitochondria and Golgi.

29. • Koch A, Yoon Y, McNiven MA, Schrader M: **A role for hFis1 in both mitochondrial and peroxisomal fission in mammalian cells**. *In Internatl. Meeting on the Topogenesis of Organellar Proteins, Ruhr Congress; Bochum, Germany*. 2004: abstract P38.
Fis1, a DLP1-interacting protein previously found on mitochondria, is also targeted to the peroxisomal membrane. It is required for peroxisome fission after tubulation, the step at which DLP1 functions, indicating that Fis1 recruits DLP1 to peroxisomes.

30. Erdmann R, Blobel G: **Giant peroxisomes in oleic acid-induced *Saccharomyces cerevisiae* lacking the peroxisomal membrane protein Pmp27p**. *J Cell Biol* 1995, **128**:509-523.

31. Smith JJ, Marelli M, Christmas RH, Vizeacoumar FJ, Dilworth DJ, Ideker T, Galitski T, Dimitrov K, Rachubinski RA, Aitchison JD: **Transcriptome profiling to identify genes involved in peroxisome assembly and function**. *J Cell Biol* 2002, **158**:259-271.

32. Tam YY, Torres-Guzman JC, Vizeacoumar FJ, Smith JJ, Marelli M, Aitchison JD, Rachubinski RA: **Pex11-related proteins in peroxisome dynamics: a role for the novel peroxin Pex27p in controlling peroxisome size and number in *Saccharomyces cerevisiae***. *Mol Biol Cell* 2003, **14**:4089-4102.

33. Rottensteiner H, Stein K, Sonnenhol E, Erdmann R: **Conserved function of Pex11p and the novel Pex25p and Pex27p in peroxisome biogenesis**. *Mol Biol Cell* 2003, **14**:4316-4328.

34. Marshall PA, Dyer JM, Quick ME, Goodman JM: **Redox-sensitive homodimerization of Pex11p: a proposed mechanism to regulate peroxisomal division**. *J Cell Biol* 1996, **135**:123-137.

35. Tam YY, Rachubinski RA: ***Yarrowia lipolytica* cells mutant for the *PEX24* gene encoding a peroxisomal membrane peroxin mislocalize peroxisomal proteins and accumulate membrane structures containing both peroxisomal matrix and membrane proteins**. *Mol Biol Cell* 2002, **13**:2681-2691.

36. Vizeacoumar FJ, Torres-Guzman JC, Tam YY, Aitchison JD, Rachubinski RA: **YHR150w and YDR479c encode peroxisomal integral membrane proteins involved in the regulation of peroxisome number, size, and distribution in *Saccharomyces cerevisiae***. *J Cell Biol* 2003, **161**:321-332.

37. Brown TW, Titorenko VI, Rachubinski RA: **Mutants of the *Yarrowia lipolytica PEX23* gene encoding an integral peroxisomal membrane peroxin mislocalize matrix proteins and accumulate vesicles containing peroxisomal matrix and membrane proteins**. *Mol Biol Cell* 2000, **11**:141-152.

38. • Vizeacoumar FJ, Torres-Guzman JC, Bouard D, Aitchison JD, Rachubinski RA: **Pex30p, Pex31p, and Pex32p form a family of peroxisomal integral membrane proteins regulating peroxisome size and number in *Saccharomyces cerevisiae***. *Mol Biol Cell* 2004, **15**:665-677.
This paper describes the genes regulating peroxisome size and number, their epistatic interactions and their roles.

39. Boukh-Viner T, Guo T, Alexandrian A, Cerracchio AG, Haile S, Milijevic S, Solomon J, Wong V, Vali H, Rachubinski RA, *et al.*: **Mechanisms of peroxisome fusion, assembly and division**. In *Internatl. Meeting on the Topogenesis of Organellar Proteins, Ruhr Congress; Bochum, Germany*. 2004: abstract L3.

40. Shimizu M, Takeshita A, Tsukamoto T, Gonzalez FJ, Osumi T: **Tissue-selective, bidirectional regulation of PEX11α and perilipin genes through a common peroxisome proliferator response element**. *Mol Cell Biol* 2004, **24**:1313-1323.

41. Li X, Gould SJ: **PEX11 promotes peroxisome division independently of peroxisome metabolism**. *J Cell Biol* 2002, **156**:643-651.

42. Schrader M, Reuber BE, Morrell JC, Jimenez-Sanchez G, Obie C, Stroh TA, Valle D, Schroer TA, Gould SJ: **Expression of PEX11β mediates peroxisome proliferation in the absence of extracellular stimuli**. *J Biol Chem* 1998, **273**:29607-29614.

43. Schrader M, Krieglstein K, Fahimi HD: **Tubular peroxisomes in HepG2 cells: selective induction by growth factors and arachidonic acid**. *Eur J Cell Biol* 1998, **75**:87-96.

44. Schrader M, Fahimi HD: **Mammalian peroxisomes and reactive oxygen species**. *Histochem Cell Biol* 2004, **122**:383-393.

45. Rottensteiner H, Hartig A, Hamilton B, Ruis H, Erdmann R, Gurvitz A: ***Saccharomyces cerevisiae* Pip2p–Oaf1p regulates**

***PEX25* transcription through an adenine-less ORE**. *Eur J Biochem* 2003, **270**:2013-2022.

46. Gurvitz A, Hiltunen JK, Erdmann R, Hamilton B, Hartig A, Ruis H, Rottensteiner H: ***Saccharomyces cerevisiae* Adr1p governs fatty acid β-oxidation and peroxisome proliferation by regulating *POX1* and *PEX11***. *J Biol Chem* 2001, **276**:31825-31830.

47. Marshall PA, Krimkevich YI, Lark RH, Dyer JM, Veenhuis M, Goodman JM: **Pmp27 promotes peroxisomal proliferation**. *J Cell Biol* 1995, **129**:345-355.

48. Lorenz P, Maier AG, Baumgart E, Erdmann R, Clayton C: **Elongation and clustering of glycosomes in *Trypanosoma brucei* overexpressing the glycosomal Pex11p**. *EMBO J* 1998, **17**:3542-3555.

49. Abe I, Fujiki Y: **cDNA cloning and characterization of a constitutively expressed isoform of the human peroxin Pex11p**. *Biochem Biophys Res Commun* 1998, **252**:529-533.

50. Eitzen GA, Szilard RK, Rachubinski RA: **Enlarged peroxisomes are present in oleic acid-grown *Yarrowia lipolytica* overexpressing the *PEX16* gene encoding an intraperoxisomal peripheral membrane peroxin**. *J Cell Biol* 1997, **137**:1265-1278.

51. Kural C, Kim H, Syed S, Goshima G, Gelfand VI, Selvin PR: **Kinesin and dynein move a peroxisome in vivo: a tug-of-war or coordinated movement?** *Science* 2005, in press.