NIH Public Access
Author Manuscript

Nat Rev Mol Cell Biol. Author manuscript; available in PMC 2014 June 17.

Published in final edited form as:
Nat Rev Mol Cell Biol. 2013 December ; 14(12): 803–817. doi:10.1038/nrm3700.

Peroxisomes take shape

Jennifer J. Smith and **John D. Aitchison**
Seattle Biomedical Research Institute, 307 Westlake Avenue North, 98109-5240, USA. Institute for Systems Biology, 401 Terry Avenue North, Seattle, Washington 98109-5219, USA

Jennifer J. Smith: jennifer.smith@systemsbiology.org; John D. Aitchison: john.aitchison@seattlebiomed.org

Abstract

Peroxisomes carry out various oxidative reactions that are tightly regulated to adapt to the changing needs of the cell and varying external environments. Accordingly, they are remarkably fluid and can change dramatically in abundance, size, shape and content in response to numerous cues. These dynamics are controlled by multiple aspects of peroxisome biogenesis that are coordinately regulated with each other and with other cellular processes. Ongoing studies are deciphering the diverse molecular mechanisms that underlie biogenesis and how they cooperate to dynamically control peroxisome utility. These important challenges should lead to an understanding of peroxisome dynamics that can be capitalized upon for bioengineering and the development of therapies to improve human health.

Numerous metabolic pathways take place within peroxisomes, most notably the β-oxidation of fatty acids and the degradation of toxic hydrogen peroxide. These organelles are remarkably diverse and, depending on the cell type and the environment, they can take on various forms and functions[1]. Consistent with this, the molecular mechanisms by which peroxisomes are formed are emerging as processes that have matching plasticity and dynamics[2]. For many years, studies in several organisms have aimed to understand peroxisome biogenesis, but they have often led to discrepancies and contradictory interpretations[3]. Efforts to reconcile these findings have shed considerable new light on how distinct aspects of peroxisome biogenesis affect their abundance and how such processes are coordinately regulated to also control the functions of these organelles. For example, it is seems that there are at least two mechanisms of peroxisome formation that are likely to be differently utilized to renew peroxisomes depending on the needs of the cell. In addition, it is becoming evident that the regulation of other aspects of peroxisome biogenesis, such as import of matrix proteins, also control their functions[2,4]. Although it has long been known that, unlike most organelles, peroxisomes can import large protein complexes, the mechanisms and implications of this amazing capability have remained mysteries. Significant advances have been made in understanding the mechanism of oligomeric protein import as well as its effects on peroxisome differentiation and the maintenance of two distinct peroxisome populations in a cell[4]. These and other exciting advances are improving

© 2013 Macmillan Publishers Limited. All rights reserved

Competing interests statement
The authors declare no competing interests.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

our understanding of the coordinated molecular mechanisms underlying peroxisome dynamics and function, and are helping to establish fundamental principles of cellular organization. In this Review, we outline distinct aspects of peroxisome biogenesis that affect the dynamics of peroxisome structure and function in various organisms, excluding plants. The discussion of peroxisome degradation (termed pexophagy) and peroxisome inheritance is limited and focuses on new insights that suggest their coordinated regulation with biogenesis (for further details of these processes, readers are referred to other reviews[5,6]).

Peroxisomes in health and disease

Peroxisomes are found in virtually all eukaryotic cells and were originally defined as organelles that contain at least one oxidase and one catalase for the respective production and decomposition of hydrogen peroxide[7]. Depending on the cell type and the environment, peroxisomes have diverse regulated functions (BOX 1), the most notable of which are related to lipid metabolism. These functions are integrated with processes in other cellular compartments, including chloroplasts, mitochondria and the cytosol, through the existence of both shared and coordinated metabolic pathways[8].

Box 1

Metabolic functions of peroxisomes

Peroxisome have diverse functions across the kingdoms (see the table), that range from the most notable and highly conserved (the β-oxidation of fatty acids paired with the degradation of H_2O_2 by catalase) to functions that are very specialized and only found in a few organisms or cell types (such as glycerol metabolism and the maintenance of cellular integrity). For example, whereas some protozoans such as *Dictyostelium discoideum* have typical peroxisomes, others, including the human pathogens *Leishmania* and *Trypanosoma* spp., have specialized peroxisomes (glycosomes) that do not seem to contain catalase, the hallmark enzyme of peroxisomes, and instead contain glycolytic enzymes (FIG. 1g). They are considered specialized versions of peroxisomes, as they share the same protein targeting and biogenesis machinery. In other examples, glyoxysomes of plants and filamentous fungi contain β-oxidation enzymes, but they also contain key enzymes of the glyoxylate cycle. Moreover, specialized peroxisomes in filamentous fungi called Woronin bodies are involved in the maintenance of cellular integrity through wound healing. Peroxisome functions in higher eukaryotes seem to be even more diverse and complex; in humans, they are involved in various aspects of human health. Of particular interest is their role in the synthesis of plasmalogens, which are enriched in the nervous, immune and cardiovascular systems and are involved in signalling and the protection of cells from reactive oxygen species (ROS) damage[148]. In addition, peroxisome proliferation controls a ROS signalling pathway in neurons that regulates energy homeostasis and feeding in diet-induced obesity[149]. They also have a newly identified role as signalling platforms in mammalian cells. Upon viral infection, mitochondrial antiviral signalling (MAVS) adaptor protein, which is located on peroxisomes, induces the expression of defence factors that provide short-term antiviral protection; this function is complemented by MAVS-mediated activation of a signalling

pathway that amplifies and stabilizes the response[150]. These and other roles emphasize the significance of peroxisomes in many complex and diverse biological processes.

Partially or exclusively peroxisomal pathways	Plants	Fungi	Protozoa	Animals
Biosynthesis				
Bile acids				✓
Hormonal signalling molecules	✓			✓
Polyunsaturated fatty acids				✓
Ether phospholipids (plasmalogens)			✓	✓
Pyrimidines			✓	✓
Purines				✓
Purine salvage			✓	
Antibiotics (penicillin)		✓		
Toxins for plant pathogenesis		✓		
Lys		✓		
Biotin	✓	✓		
Secondary metabolites	✓	✓		
Isoprenoid and cholesterol	✓			Unknown
Degradation				
Prostaglandin				✓
Amino acids		✓		✓
Polyamine	✓	✓		✓
H_2O_2 degradation by catalase	✓	✓	✓	✓
Oxidation of fatty acids	✓	✓	✓	✓
Purine	✓		✓	✓
Superoxide radical destruction by superoxide dismutase	✓		✓	✓
Glycerol metabolism			✓	
Glycolysis			✓	
Methanol degradation		✓		
Glyoxylate cycle	✓	✓		
Photorespiration	✓			
Other				
Maintenance of cellular integrity		✓		
Bioluminescence of firefly luciferase				✓
Signalling platforms in viral innate immune defence				✓
H_2O_2 signalling in hypothalamic neurons				✓

Dynamic structures and content

Consistent with their functions, the structures of peroxisomes are also diverse and dynamic (FIG. 1). Peroxisomes are 0.1–1 μm in diameter and are bound by single membranes that

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

enclose dense matrices primarily containing metabolic enzymes (and substrates and cofactors), which can sometimes form structured and electrondense crystalloid cores. Peroxisomes are usually spherical, but they can change their shape and be elongated or even form reticula in some cell types and environments[9,10] (FIG. 1a,b). In addition, they can conditionally increase in size and number (FIG. 1c,d), often in coordination with morphological changes in other subcellular compartments, including mitochondria[11], lipid droplets[12] and plasma membrane domains[13]. It is becoming clear that these dynamics do not simply increase the volume of the peroxisome to accommodate the induction of metabolic pathways; shape and size reconfigurations probably also have a more complex effect on peroxisome functions, for example altering the rates of reactions that occur within the organelles[14–16].

The enzymes contained within peroxisomal matrices vary depending on the cell type and the environment, and many have multiple functions and subcellular localizations. For example, ataxia-telangiectasia mutated (ATM), a kinase underlying the multisystem disease ataxia-telangiectasia, is both nuclear and peroxisomal[17]. Remarkably, the metabolic enzyme Gpd1 (glycerol-3- phosphate dehydrogenase 1) redistributes between peroxisomes and other cellular compartments in yeast in response to stress[18]. Another enzyme, the nicotinamidase Pnc1, is localized in the nucleus, the cytosol and in peroxisomes and extends the lifespan of yeast in response to low-intensity stress[19]. From these and other data, new communication pathways between peroxisomes and other cellular compartments are emerging that support molecular links between peroxisomes and ageing[20].

Peroxins and disease

Peroxisomal membranes contain peroxisomal membrane proteins (PMPs), which are primarily metabolite transporters and peroxins. There are 31 known peroxins that are involved in various aspects of peroxisome biogenesis, including peroxisome generation, division, as well as matrix and membrane protein import (TABLE 1). Peroxins are encoded by *PEX* genes, 14 of which have been identified in humans; these are linked to peroxisome biogenesis disorders (PBDs) (TABLE 2), which is a group of severe developmental brain disorders that typically lead to death during childhood (reviewed in REF. 8). Interestingly, mutations in three different *PEX* genes have recently been found to underlie late-onset or slowly progressing neurological disorders[21–24], and similar α-synuclein toxicity and lipid metabolism defects have been found in both Parkinson's disease and PBDs[25]. Moreover, a correlation has been made between the level of peroxisome proliferation in hippocampal neurons and the protection from β-amyloid neurodegeneration, a process that is linked to Alzheimer's disease[26]. These data, together with the emerging roles of peroxisomes in cellular ageing[20], highlight the importance of understanding peroxisome biogenesis for human health and ageing and, in particular, their roles in the advancement and treatment of neurological disorders. Furthermore, the fact that far fewer peroxins have been identified in humans than in simple model organisms (TABLE 2) suggests that there are several human peroxins that have yet to be discovered and that these may underlie disorders that are perhaps less severe or have a later onset in comparison to typical PBDs. One approach that holds promise for revealing elusive human *PEX* genes is the development and screening of

model systems for peroxisome biogenesis in complex multicellular systems such as that developed in *Drosophila melanogaster*[27].

Protein import into peroxisomes

Mechanisms of protein targeting and import into peroxisomes are fascinating and important aspects of peroxisome biogenesis. This trafficking involves the integration of classic themes in protein sorting with other mechanisms that are rarely used in other compartments, giving exceptional capabilities to peroxisomes. Consistent with the involvement of multiple mechanisms in peroxisome formation, there are various trafficking routes for both matrix and membrane protein import. These may be important for robustness or adaptation[28] but in some cases may provide a mechanism for selective targeting to specific classes of peroxisomes[29]. The targeting signals and mechanisms for matrix and membrane protein import are distinct and will be discussed separately.

Matrix protein import

Matrix proteins are post-translationally targeted to peroxisomes from the cytosol by peroxisomal targeting signals (PTSs). These signals include the predominantly used PTS1 and the less prevalent PTS2, which are recognized by the soluble import receptors PEX5 and PEX7, respectively. Import is achieved by a group of up to 13 membrane-associated peroxins that collectively make up the matrix protein import machinery, which functions to dock cargo-bound import receptors at the peroxisomal membrane, translocate cargo into peroxisomes and export receptors back to the cytosol (FIG. 2a; TABLE 1). The process of cargo translocation is remarkable, as peroxisomes have the ability to import very large protein oligomers and even 9 nm gold particles[30]. However, the mechanism for this remained an enigma for several years because, despite this import capacity, peroxisomes are impermeable to all but small metabolites[31] and no pore-like structure has ever been observed[32]. These data led to several theories based on transient import structures, including the transient pore hypothesis and the membrane invagination model[33].

Considerable progress has now been made to characterize the PTS1-dependent translocation mechanism, and a four-step model has been proposed[34,35]: first, cargo is bound by the predominantly soluble import receptor, PEX5, in the cytosol; second, the cargo–receptor complex attaches to peroxisomes through the docking complex on the peroxisomal membrane; third, PEX5 integrates into the membrane to form a transport channel through which cargo passes; and last, PEX5 receptors are removed from the membrane and recycled for another round of import in an energy-dependent fashion by a complex called the exportomer[35]. Thus, both the import and recycling steps are important for this recycling-driven import.

Strong data that support the translocation step include a biochemical analysis of the properties of Pex5–protein A complex that was affinity purified from solubilized yeast membranes and reconstituted into liposomes[36]. Analysis of the liposomes using a planar lipid bilayer technique showed that Pex5 integrates into the membrane and forms a gated ion-conducting channel together with the PMP Pex14 through which cargo passes. The data indicated that the pore is made up of Pex5 oligomers, is dynamic and can adopt both low

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

and high conductance forms, which correspond to inactive and active states. This model was built on previous data in yeast showing that import of Pex8 into peroxisomes requires only PTS receptors and Pex14 but no other components of the import machinery[37], and that PEX5 integrates into membranes in yeast and mammalian cells[38,39] (reviewed in REF. 32). This model is also supported by the fact that PEX5 and PEX14 each form homo-oligomers[40,41] and interact with one other[42], and that many matrix proteins are imported in an oligomeric state[43]. The model further predicts that oligomerization of cargo can improve the efficiency of import by bringing multiple receptors into close proximity to form a transport channel, an idea that was previously proposed in the preimplex hypothesis (REF. 44). This idea is supported by a recent analysis of specialized peroxisomes called Woronin bodies in *Neurospora crassa,* showing that oligomerization is in fact necessary for the efficient import of the PTS1-containing matrix protein HEX[4], but this does not seem to hold true for all oligomeric matrix proteins[45,46]. Additionally, the model can explain the selective permeability of peroxisomes; as the receptor forms the import channel, it provides a natural means of tailoring the size of the active pore to the size of the cargo, assuming that the number of cargo molecules is proportional to the number of import receptors that form the channel.

The last step of import, receptor recycling, would require that the receptor is either recycled back to the cytoplasm for further rounds of import or that it is directed to the proteasome for destruction when receptor recycling is dysfunctional (by a pathway called receptor accumulation and degradation in the absence of recycling (RADAR)). Recycling and RADAR are controlled by different receptor ubiquitylation events that are mediated by ubiquitin ligases (RING finger group proteins) and conjugating enzymes that are associated with the exportomer[47–49]. Both pathways involve the ATP-dependent extraction of the receptors by the PEX1–PEX6 AAA-type ATPase complex[50,51], which is recruited to the cytoplasmic face of the peroxisomal membrane by PEX26 (Pex15 in *S. cerevisiae*)[52,53]. Interestingly, this recycling-driven import mechanism is mechanistically similar to endoplasmic reticulum-associated degradation (ERAD), which removes misfolded proteins from the ER in a manner that also involves ubiquitylation of the target and ATP-dependent protein removal from the membrane by an AAA-type ATPase[54–56]. It has been proposed that this energy-dependent export drives the import of matrix proteins into peroxisomes, a hypothesis that is termed export-driven import[54]. However, import is still possible in cells with blocked export as long as the RADAR pathway is functional, but these cells have a growth defect on peroxisome-requiring medium[57]. This suggests that receptor removal by recycling or degradation drives the import of matrix proteins (provided that the receptors are not limiting). This hypothesis perhaps explains the involvement of most components of the import machinery in receptor recycling functions (FIG. 2a; TABLE 1); in yeast, nine proteins mediate receptor ubiquitylation or extraction, whereas only four are involved in translocation.

Less is known about the second import route for matrix proteins (that is, PTS2-dependent import), but it seems to converge with the PTS1-dependent pathway at the docking complex[58] and to involve similar receptor recycling and degradation steps that drive cargo translocation[57,59]. It is active in most organisms studied, but is generally used by few

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

cargoes. In mammalian cells, PTS2- and PTS1-dependent import are coupled, and PTS2-mediated translocation requires a long isoform of PEX5 (also known as PEX5L or PEX5l) that physically associates with the PTS2 receptor, PEX7. In lower eukaryotes, import of PTS2-containing cargo is independent of Pex5, and instead requires a Pex7-binding co-receptor that has sequence similarity to Pex5 (Pex20 in most fungi studied[28]) (TABLES 1,2). As PTS-containing proteins can be imported as protein complexes, a third import route involves a physical association ('piggybacking') with another protein that contains a PTS[60–62], which offers an explanation for why some matrix proteins do not have recognizable PTSs.

Significance of oligomeric import

Complementing this emerging model of how large protein complexes are imported across the peroxisomal membrane is the equally interesting question of why complexes rather than individual proteins are imported into peroxisomes. One possible reason for oligomeric import is efficiency; in the case of HEX, a highly abundant cargo in *N. crassa*, oligomerization is necessary for its efficient import. In addition to this, preventing HEX oligomerization seems to saturate the PTS1-dependent import pathway[4], which suggests that import of oligomers is necessary to maximize the general import capacity of peroxisomes and perhaps explains why oligomeric import into peroxisomes is so widespread[43]. A second role of oligomeric import may be to enable peroxisome differentiation[4]. Import of oligomeric HEX induces peroxisome differentiation through a positive feedback mechanism. Its oligomeric state may promote nonuniform import, leading to differentiation of only a subset of peroxisomes. Self association may also be important for the second step of differentiation, asymmetric fission, which generates two classes of peroxisomes, those with and without HEX. Finally, oligomeric import may be important for proteins that do not properly fold in the lumen of peroxisomes. In support of this, in *Hansenula polymorpha*, inhibiting folding of catalase in the cytosol before import into peroxisomes by reducing its residence time in the cytosol results in the accumulation of inactive catalase protein aggregates inside peroxisomes[63] (FIG. 1e,f).

Dynamic regulation of import

The regulation of matrix protein import in response to the cell state or the environment is emerging as a mechanism to control peroxisome functions. Evidence for this extends beyond transcriptional responsiveness of components of the import machinery[64]; it has recently been shown that the binding of the import receptor Pex5 to cargoes is redox regulated (FIG. 2a) and that changing the redox balance between peroxisomes and the cytosol can disrupt import[65]. Intriguingly, import of individual cargoes can also be conditionally regulated: in yeast, Gpd1 conditionally redistributes among the cytosol, peroxisomes and the nucleus in response to osmotic stress, and the regulation of this distribution involves conditional phosphorylation near its PTS2 (REF. 18). Further exploration of these important mechanisms will be a key step to understanding the dynamics of peroxisome structure and function.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Membrane protein import

Most PMPs are synthesized on free polysomes in the cytosol and post-translationally inserted into peroxisomal membranes by one of two pathways: direct targeting to peroxisomes (class I proteins) (FIG. 2b); or targeting via the ER (class II proteins)[66]. Class I PMPs are targeted to peroxisomes through the recognition of a membrane PTS (mPTS) in the cytosol by the shuttling receptor PEX19 (REF. 67). PEX19–cargo complexes are then recruited to peroxisomes by membrane-bound PEX3 (or PEX16 in mammalian cells), and cargo PMPs are inserted into the membrane[67,68] and assembled into complexes by mechanisms that are mediated by the chaperone activity of PEX19 (REF. 69).

The mechanism of import for class II PMPs has been most extensively studied in yeast and is shown in FIG. 3. In yeast, PMPs are first inserted into the ER membrane using the same machinery that promotes transit of secreted proteins, including the Sec61 translocon[70,71] and the GET complex[71,72]; however, PMPs that traffic through the ER do not contain canonical cleavable signal peptides, and only one PMP (PEX16 in mammalian cells) has been shown to be co-translationally inserted into the ER membrane[73]. Exit from the ER involves: the formation of a specialized region of the ER that is called the peroxisomal ER or the preperoxisomal compartment[71,73–75]; and subsequent budding of preperoxisomal vesicles from this region. Surprisingly, these processes seem to require a unique set of factors that have not been characterized previously in secretion, including Pex3 and Pex19 in yeast[71] and PEX16 in mammalian cells[73], but studies in mammalian cells show that peroxisome formation does not require the vesicle coat protein complex COPII[76,77]. This is supported by data from recently developed *in vitro* budding assays in yeast, which have shown that trafficking of Pex15, Pex11 and Pex3 depends on Pex19, ATP and the cytosol but is independent of COPII[58, 78]. Other known components of the secretory pathway may be involved in budding of preperoxisomal vesicles but eluded detection by functional assays[79,80] because they are either functionally redundant or essential[78]. Consistent with this idea, a screen specifically targeting essential yeast genes showed that three genes involved in ER trafficking (*SEC20*, *SEC39* and *DSL1*) are also important for peroxisome biogenesis[81]. Further investigation of these candidates and analysis of essential genes on a broad scale should help to gain mechanistic insights into trafficking of PMPs and will potentially identify peroxisome-specific vesicle coat proteins.

Although it was originally proposed that direct targeting was the most prominent targeting mechanism, recent data indicate that the ER route may be more widespread than previously thought[71,82]. Live-cell imaging analysis of a comprehensive set of PMPs (class I and class II proteins) showed trafficking through the ER in wild-type yeast cells and during *de novo* generation in the absence of pre-existing peroxisomes[71]. In other yeast studies, Pex11 and Pex15, which contain class I-type mPTSs, were shown to traffic through the ER in cell-free vesicle-budding reactions[58,78]. These observations have not yet been reconciled with those from earlier work, but the discrepancy may have arisen because most evidence in favour of direct targeting has been acquired in mammalian cells, whereas ER trafficking data has been obtained primarily from yeast, many species of which do not express Pex16. Alternatively, there could be two mechanisms of PMP targeting that are differently used depending on the cellular environment and the dominant biogenesis pathway under that specific condition.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Two mechanisms of peroxisome biogenesis

The origin of peroxisomes has been a topic of debate for many years[3]. The most recent data, collected primarily using live-cell imaging with fluorescent reporters, indicate the existence of two pathways of peroxisome biogenesis: peroxisomes can be generated *de novo*, whereby preperoxisomal vesicles bud from the ER[75,83] and subsequently fuse with each other to form mature peroxisomes[82]; and pre-existing peroxisomes can produce new peroxisomes through growth and division (fission), using new proteins and lipids which are supplied from the ER in the form of vesicles[74] (FIG. 3). Although both pathways contribute to the formation of new peroxisomes, they are distinct; *de novo* generation has slower kinetics but results in peroxisomes that contain all ‘new’ material, whereas growth and division is faster, but requires the presence of pre-existing peroxisomes[74].

Peroxisome biogenesis through growth and fission

It is well established that peroxisomes undergo fission mediated by PEX11, which is found in all organisms tested, and often has multiple isoforms within an organism that can have conditional activities or roles that are not related to fission (TABLE 1). Fission also involves factors that have established roles in mitochondrial fission[84,85], including dynamin-related proteins (DRPs; also known as dynamin-like proteins (DLPs) in mammals)[86,87] and DRP-interacting proteins, such as mitochondrial fission 1 (FIS1)[88]. The molecular mechanisms of peroxisomal fission are becoming clearer, and a model is emerging (FIG. 3) that seems to be similar in yeast and mammalian cells[89]. First, Pex11 (PEX11β in mammalian cells) becomes activated and mediates tubulation of peroxisomes[90,91], and membrane-anchored DRP-interacting proteins are enriched in the elongated membrane. Next, the membrane is constricted and DRPs, recruited from the cytosol by DRP-interacting proteins[92,93], promote membrane scission to form new peroxisomes[89]. The regulation of these molecular events is not well characterized, but it has been shown that in yeast, phosphorylation of Pex11 (REF. 94) regulates its interaction with Fis1 (REF. 95). In addition, there is some evidence that the oligomeric state of Pex11 (monomeric versus dimeric) is redox-sensitive and that this could coordinate division with the levels of oxidative metabolism within maturing peroxisomes[96]. The DRP-mediated regulatory mechanisms have not yet been demonstrated, but considering that DRPs and DRP-interacting proteins have dual roles in peroxisome and mitochondrial fission, they have the potential to facilitate the coordination of peroxisome and mitochondrial proliferation[11,97], or the coordination of peroxisome proliferation with mitochondrial function[98,99].

Fission is coordinated with the growth of peroxisomes via expansion of the peroxisomal membrane. This is achieved by the transfer of proteins and membrane to mature peroxisomes from the ER by vesicular transport[74]. These vesicles are distinct from the ER-derived vesicles involved in the *de novo* generation of peroxisomes because vesicles involved in the *de novo* process cannot fuse with mature peroxisomes[82]. The molecular mechanism of vesicular transport to mature peroxisomes has not been fully elucidated, but in yeast, the vesicles contain the multifunctional peroxin Pex3. It will be interesting to learn the role of Pex3 in this process and to address whether peroxins implicated in vesicle fusion (Pex1 and Pex6) (TABLE 1) are also involved.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

De novo peroxisome biogenesis

Peroxisomes can also be formed *de novo* from the ER by a second vesicular transport mechanism. This assembly route was first identified in *Yarrowia lipolytica* and involves the generation and fusion of distinct classes of preperoxisomal vesicles with each other[100]. The mechanism was recently characterized in *Saccharomyces cerevisiae* and involves the production and heterotypic fusion of two classes of preperoxisomal vesicles, each containing a different subset of peroxisomal proteins[82]. The classification of these vesicles revealed the presence of an elegant sorting mechanism that suppresses the import of matrix proteins into peroxisomes until budding is complete and preperoxisomal vesicles have established autonomy from the ER (FIG. 3). This process requires sorting of the subcomplexes of the import machinery (proteins of the docking complex and the RING ubiquitylation complex) (TABLE 1) into two separate preperoxisomal vesicles. After egression, pairs of these different vesicles fuse to form import-competent peroxisomes. Fusion seems to be selective, and preperoxisomal vesicles cannot fuse with mature peroxisomes, a restriction that might ensure the correct molar ratios of components of the import machinery in mature peroxisomes.

This newly identified process requires specificity during both vesicle formation and fusion, which has not yet been characterized, but mechanistic insight can be gleaned from the components of each class of vesicle. The two AAA-type ATPase peroxins, Pex1 and Pex6, are differently represented in each vesicle class, and they might mediate heterotypic fusion of the vesicles to form mature peroxisomes[82], which is consistent with their previous implication in this process[101]. Perhaps their selective enrichment in different classes of vesicle provides specificity to the fusion event, but how they are differently sorted has yet to be determined. The characterization of how these and other PEX proteins affect this biogenesis pathway is an important challenge, as multiroute trafficking mechanisms are involved in the assembly of other functional complexes[102–104] and thus may be a widespread mechanism that is fundamental for maintaining biochemically distinct compartments within the cell[82].

Coordination of division with *de novo* generation of peroxisomes

A question of major interest is how *de novo* generation and fission are coordinately and dynamically regulated to control peroxisome abundance and function. Given the conditional roles of peroxisomes in carbon source metabolism and in the production of reactive oxygen species, which can damage proteins and lipids, it is likely that the source of new peroxisomes are affected by cellular states and that this can be influenced by both past and present environmental conditions. Perhaps these influences can explain the ongoing discrepancies about the origin of peroxisomes. For example, two recent studies come to the contradictory conclusions that peroxisomes are derived *de novo*[82] and that they are derived exclusively from pre-existing peroxisomes[74] in wild-type yeast, despite the fact that both studies measured yeast grown under fermentative conditions. There are similar contradictions about the origins of mammalian peroxisomes, which have been found to form *de novo* in cells with pre-existing peroxisomes[73] despite previous evidence to the contrary[105,106]. Considering these inconsistencies, future studies that measure how the

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

activities of *de novo* generation and fission are coordinated and affected by the cell state will be very valuable.

A model in *H. polymorpha* that genetically separates *de novo* biogenesis from fission can be useful in this regard[107]. It takes advantage of the role of Pex25 in the *de novo* generation of peroxisomes[107,131], and is based on a *pex25 pex11* double-deletion strain, which has no peroxisomes because both *de novo* generation of peroxisomes and fission are blocked. The restoration of *de novo* generation in this strain by reintroducing *PEX25* resulted in peroxisome formation. By contrast, the reintroduction of *pex11* into this strain, which restored fission in the absence of pre-existing peroxisomes, did not result in the production of peroxisomes, even though the genetically identical *pex11*-deletion strain (without prior elimination of peroxisomes) has peroxisomes. These data indicate that in *pex11* and *pex25* single-deletion strains, the only active biogenesis pathways are *de novo* generation and fission, respectively, and they also suggest the utility of the strains for interrogating each pathway independently to determine how they respond to genetic and environmental perturbations. Such studies will be important for understanding the regulation and coordination of the two pathways.

Peroxisome motility and inheritance

Directed movement of peroxisomes along actin cables in yeast or microtubules in mammalian cells is an important aspect of peroxisome biogenesis. Considering the implications of peroxisomes in neuronal pathologies[25,108], peroxisome positioning is likely to have additional importance in specific cell types such as neurons, which are extremely long. In mammalian cells, movement along microtubules disperses peroxisomes after fission[89], and in yeast, peroxisome motility is necessary for inheritance or directed movement of peroxisomes from mother to daughter during cell division, which is paired with the retention of peroxisomes in both cells to ensure equitable distribution of peroxisomes between them. This process involves the attachment of peroxisomes to actin cables by interaction of myosin II, a class V myosin, with the peroxisome-specific factor Inp2 (inheritance of peroxisomes 2) for peroxisome movement into the bud[109], and anchoring of peroxisomes in the mother and bud through Inp1 (REF. 110). Actin-based movement is also required in yeast for pexophagy (selective autophagic degradation of peroxisomes)[111]. PEX14 is emerging as a key player in these processes: in mammalian cells, it is necessary for peroxisome movement through a direct interaction with tubulin, which comprises microtubules[112]; and in yeast it interacts with the actin-binding protein dynamin 2 (REF. 113) and is necessary for macropexophagy[114].

Regulation of peroxisome dynamics

Evidence indicates that changes in the shape of a compartment (including both volume changes in vesicles and connectivity changes in a reticulum) influence the rates of the embedded chemical reactions[15,115]. Therefore, dynamic changes in peroxisome structure may actively regulate peroxisomal metabolic processes[158]. This is supported by an innovative study showing that overproduction of Pex11, which results in the increased abundance and decreased size of peroxisomes, increases penicillin production in the fungi

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Penicillium chrysogenum without affecting the levels of peroxisomal enzymes involved in the generation of penicillin[14]. Thus, understanding how the dynamics of peroxisome structure are regulated and how they can influence peroxisome function are important areas of study that can advance therapeutic and bioengineering strategies. Here, we discuss insights that have been gained into how peroxisome proliferation and morphogenesis are regulated.

Control of peroxisome size and number

In the absence of a stimulus for peroxisome proliferation, peroxisomes seem to be in homeostasis and multiply at a rate that is comparable to the cell cycle[74]; but, in the presence of an environmental cue such as fatty acids, peroxisomes increase in size and number (FIG. 1c,d). The control of peroxisome dynamics is achieved in part at the level of transcription. Fatty acid-responsive transcription factors induce the expression of specific peroxins and enzymes during peroxisome proliferation[64,116,117], including peroxins of the PEX11 family and peroxisomal metabolic enzymes, both of which affect peroxisome size and number in yeast and human cells[89,118–120]. Such responses probably also involve signal transduction pathways, as several proteins seem to be phosphorylated during peroxisome proliferation in yeast[121]; however, except for a few examples, such as the recent demonstration that Pex11 phosphorylation controls peroxisome fission in yeast[94,95], these responses are largely unexplored. Other signalling mechanisms that are beginning to emerge include a metabolic signal from inside peroxisomes that regulates fission[122] and a peroxisome–ER tether involving Pex3 and the inheritance factor Inp1 that senses peroxisome number during cell division to control peroxisome abundance[123]. Interestingly, a second peroxisome–ER association has been implicated in the control of peroxisome proliferation in yeast, which involves the interaction of Pex23-family peroxins with reticulon homology domain-containing proteins at specific subdomains of the ER[124].

It is likely that the regulation of peroxisome size and number is complex and extends beyond the control of peroxisome proliferation (by fission and *de novo* generation). There is evidence that these processes are coordinated with others, including the degradation and segregation of peroxisomes into new cells. Pex3 may be key in this coordination[125]; in addition to its roles in peroxisome biogenesis, it is essential for the degradation of peroxisomes by pexophagy[125–128] and the segregation of peroxisomes into daughter cells during division[129,130]. Additional candidate coordinators of these processes include other multifunctional peroxins, such as the Pex11 family proteins, which mediate fission and *de novo* formation[107,131], as well as retention of peroxisomes during inheritance[132]; Pex19, which affects egression of preperoxisomal vesicles from the ER[71] and peroxisome inheritance in yeast[133]; and PEX14, which is involved in matrix protein import and also pexophagy in yeast[114,134] and peroxisome motility in mammalian cells[112]. An important outstanding challenge is to determine how these multifunctional proteins are involved in the coordination of *de novo* generation and division with inheritance and turnover to control peroxisome size and number.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Control of peroxisome morphogenesis

Peroxisome elongation is a transient stage of peroxisome fission. However, widespread and sustained elongation of peroxisomes and even a complex peroxisome reticulum have been observed in some mammalian cell types[9,135,136]. For example, in COS7 cells (African green monkey kidney cells), many peroxisomes are spherical, but they are also frequently observed in an elongated form[10,137] (FIG. 1). Tubulation and elongation are regulated, as these morphologies can be induced by stimuli such as partial hepatectomy[136] and exposure of cells to growth factors or polyunsaturated fatty acids (PUFAs)[137]. These observations reiterate that morphogenesis may be a mechanism for altering the activity of peroxisomal metabolic pathways and thus warrants further investigation. We do not know the molecular mechanisms of morphogenesis, but as peroxisome elongation associated with peroxisome division requires PEX11, it is a good candidate.

Peroxisome differentiation

The presence of different subtypes of an organelle is not a new concept. For example, the ER has several subtypes with specialized functions, including rough, smooth and transitional ER. Depending on the organism and cell type, peroxisomes have different appearances, contents and functions. However, for certain cell types such as those in liver and kidney tissues, subpopulations of peroxisomes can have distinct compositions and abundances within the same cell[138], and the different populations seem to be derived from the same parent organelle[136]. This raises the question of how a cell can produce and maintain different subpopulations of an organelle in the same cell type. Indeed, this is a fundamental question in cell biology. Studies using two model systems have shed light on this. In the first study, the fate of intraperoxisomal aggregates of a mutant form of catalase was followed in *H. polymorpha*[139]. This study identified a pathway, whereby aggregates are sequestered into a distinct subpopulation of peroxisomes (FIG. 1e,f), which are then degraded by autophagy. This process involves asymmetric fission of peroxisomes to isolate the aggregates, which is mediated by fission components, Pex11 and the DRP Dnm1.

Another study characterized the differentiation of peroxisomes into Woronin bodies in the multicellular filamentous fungi *N. crassa*[4]. These organelles are a remarkable, specialized subtype of peroxisomes that contain a crystalline hexagonal core composed of a self-assembling aggregate of HEX proteins. The organelles are localized near cell junctions and act as plugs during wound repair to block the open channel between two cells when one is damaged, thereby preserving the other. Despite the use of common import mechanisms, Woronin bodies are less abundant than peroxisomes[140], and the two subtypes have distinct protein compositions. These organelles seem to form from a subset of peroxisomes via an interesting positive feedback mechanism that is based on the import of HEX oligomers that promotes differentiation[4]. As HEX physically associates with the exportomer component PEX26, import of oligomeric HEX promotes PEX26 enrichment in membranes of peroxisomes undergoing differentiation; this further promotes the import of additional HEX oligomers into differentiating peroxisomes. These peroxisomes then undergo asymmetric fission to form two physically and functionally distinct compartments: a Woronin body that contains the HEX aggregate; and a parent peroxisome that is enriched for PEX26. As

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

PEX26 functions specifically in receptor recycling (FIG. 2a; TABLE 1), this proposed feedback mechanism is consistent with the export-driven import hypothesis.

The importance of protein aggregation in the biogenesis of both peroxisome subtypes discussed is interesting and suggests the existence of shared underlying mechanisms. The implication of peroxins in peroxisome differentiation is an exciting finding that implies that this process is coordinated with other aspects of biogenesis. These new links may lead to a better understanding of the apparent interconversion of glyoxysomes and peroxisomes during germination and maturation of some plant seedlings[141], and of the existence of peroxisome subtypes in various human cells[138]. Further studies in this area may be useful to engineer peroxisomes with new capabilities and for drug development applications.

Perspectives

Considerable progress has been made to give us a much clearer picture of how peroxisomes are formed and maintained. We owe much of this to the numerous model systems available. Studies in these diverse contexts have revealed both generalizable themes to establish robust mechanistic models of peroxisome biogenesis, as well as important distinctions between organisms that cannot only be used to gain insights into evolution but can also be capitalized on for developing therapies. For example, drugs can be targeted to disable peroxisome formation in pathogens without detrimental effects on human hosts[142]. However, beyond typical model systems, peroxisome research is notable for its exploitation of numerous, very closely related yeast model systems. Although this is a remnant of early searches for strains that can grow well on fatty acids as a sole carbon source — to thereby facilitate genetic screens for *PEX* genes — these models became mainstays and naturally led to multiple analyses of the same genes and proteins in very similar systems. Through this collective approach, many mechanistic models have been reinforced, but a surprising number of them have been shown to be inconsistent across species and even within the same organism.

From these discrepancies, it is evident that peroxisome dynamics are complex and are regulated by the coordinate activities of several intrinsic processes (including *de novo* biogenesis, fission, import, protein degradation and inheritance), as well as several other processes elsewhere in the cell[112,113,143,144]. The mechanistic details of these processes are developing rapidly, but the details of how each process responds to the cell state and the environment are emerging more slowly. Important future challenges will be to elucidate these dynamic regulatory pathways and to decipher how the various processes that affect peroxisome dynamics are coordinated. A developing idea of significance is that the coordinate regulation and ultimately the control of peroxisome structure and function are achieved in part by multifunctional peroxins[28,125]. Characterizing the precise roles of these proteins in this coordinate regulation will be an important task. In addition, linking these regulatory mechanisms with other cellular processes and structures is key and will benefit from the incorporation of systems approaches. Data sets connecting peroxisome dynamics with other cellular processes have been generated in yeast[64,79,80,116,145] and should help to build predictive network models of widespread peroxisome communication and identify areas for further investigation.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Systems approaches could also be applied in a second way: to generate detailed mechanistic models of peroxisome biogenesis that are comparable to those generated for endosome biogenesis[146] and vesicle fusion[147] (BOX 2). Exciting new discoveries have linked peroxins to the direct regulation of peroxisome function[4,14]. Because these studies have shown that the receptor recycling component PEX26 controls differentiation of peroxisomes into two subtypes with different functions and that Pex11 can regulate penicillin production, it is possible that these findings could be combined into mathematical models of peroxisome biogenesis. These models could be used as tools to control peroxisome function for bioengineering and drug development. Building such models will be challenging and will require a more complete understanding of biogenesis through the comprehensive identification and characterization of all proteins involved. In addition, modelling will require: data sets from genome-wide screens aimed at identifying essential genes that are involved in biogenesis, as was recently performed on a smaller scale[81]; and the characterization of all peroxins through various global assays, which will need to be conducted under carefully controlled and matched conditions. Modelling of biogenesis is an ambitious aim, but our growing understanding of the organelle and its components are leading us towards this outstanding goal.

Box 2

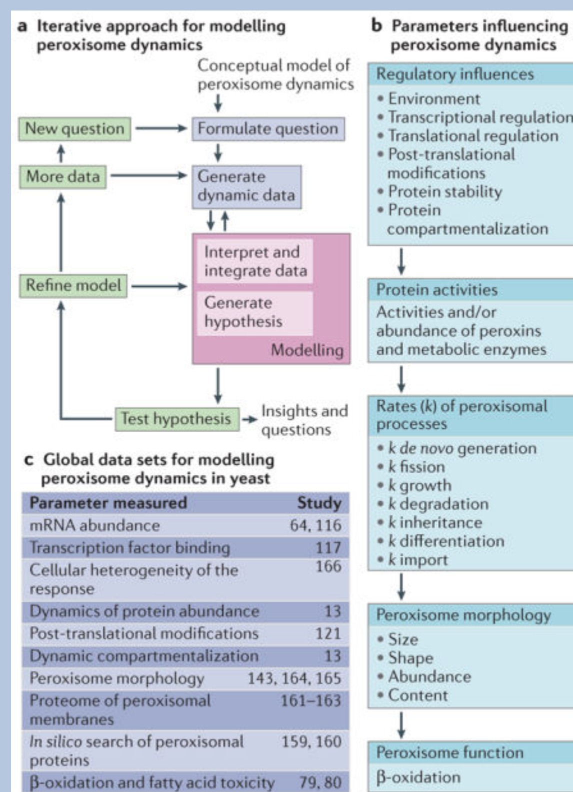
Studying peroxisome dynamics

Systems approaches have been applied to understand and predict peroxisome development in yeast (see the figure, part **a**). These studies have primarily focused on modelling the structure and function of transcriptional regulatory networks[116,117,151–154], largely because the principles of eukaryotic transcription are well understood and large-scale data sets that predict transcription factor activities and targets can be obtained with relative ease. In one such approach, kinetic modelling, ordinary differential equations that underlie a biological process are generated, and the process is simulated using both estimates and known values of variables (including transcript and protein levels, protein activities and reaction rates). The models are iteratively refined to recapitulate known responses and, once optimized, they can be used to generate a testable hypothesis on the behaviour of the system under new biological conditions[153–157]. In another approach, a global environment and gene regulatory model was generated from a compendium of ~1500 public gene expression data sets that were generated under various conditions. This model accurately predicts the regulatory and gene expression changes in response to new stimuli[145] and was used to gain new mechanistic insights into the signalling and regulatory networks that control proliferation of peroxisomes in response to oleic acid.

Theoretically, similar approaches can be applied to develop cell biological models (beyond transcriptional regulatory networks) that describe the diverse processes affecting peroxisome dynamics (including *de novo* generation, fission or import of matrix proteins)[158], and eventually even link them to peroxisome function (see the figure, part **b**). This undertaking is challenging because these applications require a good understanding of how each protein affects biogenesis, as well as global data sets of protein levels and conditional activities (through, for example, protein modifications, subcellular locations and other modifiers). Some data sets that would be useful for such

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

modelling in yeast are accumulating (see the figure, part **c**). For example, condition-specific subcellular protein distribution and abundance have been globally analysed in the presence and absence of fatty acids, which induce peroxisome proliferation[13]. Data sets have been generated that predict[159, 160] and characterize[161–166] the peroxisomal proteome. In addition, a large-scale data set of protein phosphorylation in the absence and presence of peroxisome proliferation has been generated[121], as well as global data sets of peroxisome-related phenotypes of gene-deletion strains[79,80,164–166]. However, there are significant hurdles impeding the modelling of peroxisome dynamics, including the incomplete understanding of mechanistic details and the apparent sensitivity of each pathway to subtle changes in growth conditions or cell state, which have not yet been characterized. It is evident that data sets used for modelling would have to be carefully matched with the growth condition and cell state, and currently available data sets do not meet this criterion. This is an important challenge for future studies.



Parameter measured	Study
mRNA abundance	64, 116
Transcription factor binding	117
Cellular heterogeneity of the response	166
Dynamics of protein abundance	13
Post-translational modifications	121
Dynamic compartmentalization	13
Peroxisome morphology	143, 164, 165
Proteome of peroxisomal membranes	161–163
In silico search of peroxisomal proteins	159, 160
β-oxidation and fatty acid toxicity	79, 80

Acknowledgments

The work in the authors' laboratory was supported by the US National Institutes of Health Grants P50 GM076547,, U01GM098256, U54GM103511 and GM075152. The authors thank the Luxembourg Centre for Systems Biomedicine, the Life Sciences Discovery Fund and the University of Luxembourg for support.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Glossary

Peroxisome inheritance	The active recruitment of peroxisomes from a mother cell to a daughter cell during cell division, and the retention of peroxisomes in the mother cell and the bud to ensure equitable distribution of peroxisomes between the two cells
Peroxins	Proteins that are encoded by *PEX* genes and that are involved in peroxisome biogenesis (excluding transcriptional regulators)
***PEX* genes**	Genes encoding proteins that are involved in peroxisome biogenesis (excluding those involved in transcriptional regulation). The numbers reflect the order in which they were identified
Peroxisome biogenesis disorders, (PBDs)	A group of developmental brain disorders with a prevalence of 1:50,000. These disorders are caused by mutations in peroxin (*PEX*) genes which lead to dysfunctional peroxisome biogenesis. PBDs, or Zellweger syndrome spectrum (ZSS), include, in decreasing order of severity, Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD) and infantile refsum disease (IRD)
PTS1	The carboxy-terminal peroxisomal targeting signal 1 (with the sequence (Ser/Ala/Cys)(Lys/Arg/His)(Leu/Met/Ile)) is found on most matrix proteins
PTS2	The peroxisomal targeting signal type 2 (with the sequence (Arg/Lys)(Leu/Val/Ile) $(Xaa)_5$(His/Gln)(Leu/Arg); where Xaa represents any amino acid) is located near the amino terminus of some peroxisomal matrix proteins. It is found much less commonly than PTS1 motifs
Transient pore hypothesis	A model of matrix protein import into peroxisomes. It involves the transient existence of a protein- conducting translocon that assembles after docking of the receptor–cargo complex to peroxisomes and disassembles after translocation
Membrane invagination model	A model of matrix protein import into peroxisomes. At the site where receptor–cargo complexes dock on the surface of a peroxisome, the membrane invaginates and ‘pinches off’ to form an intraperoxisomal vesicle that is surrounded by a single membrane which is later degraded to release its contents into the peroxisome
Preimplex hypothesis	A model of the early steps of matrix protein import into peroxisomes. Multiple peroxisomal PEX5 receptors interact with multiple cargoes in the cytoplasm to form so-called preimplexes, which are necessary for the efficient import of cargo into peroxisomes
RING	A zinc-finger-type domain that is found in many proteins involved in the ubiquitylation pathway, including RING finger group

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

	proteins in peroxisomal membranes (peroxin 2 (PEX2), PEX10 and PEX12)
AAA-type ATPase	A large family of ATPases, including peroxin 1 (PEX1) and PEX6, that contain an ATPase domain. These proteins can drive remodelling or translocation of macromolecules through ATP hydrolysis
Membrane PTS	(mPTS). A targeting signal of peroxisomal membrane proteins. The consensus sequence is not well defined and may be discontinuous. It can consist of basic amino acids that have been predicted to form an α-helix that is either adjacent to a transmembrane segment or in a matrix-facing loop
Peroxisomal ER	Subdomain of the endoplasmic reticulum that is the site of peroxisome formation
Preperoxisomal vesicles	Vesicles that have budded from the endoplasmic reticulum and are destined to become mature peroxisomes
Mature peroxisomes	A functional peroxisome that is capable of matrix protein import
Dynamin-related proteins (DRPs)	Primarily cytosolic GTPases that are involved in membrane fusion and fission. They are recruited to peroxisomal membranes by tail-anchored membrane receptor proteins called DRP-binding proteins

References

1. Islinger M, Grille S, Fahimi HD, Schrader M. The peroxisome: an update on mysteries. Histochem Cell Biol. 2012; 137:547–574. [PubMed: 22415027]
2. Pieuchot L, Jedd G. Peroxisome assembly and functional diversity in eukaryotic microorganisms. Annu Rev Microbiol. 2012; 66:237–263. [PubMed: 22994494]
3. Kunau WH. Peroxisome biogenesis: end of the debate. Curr Biol. 2005; 15:R774–R776. [PubMed: 16169481]
4. Liu F, Lu Y, Pieuchot L, Dhavale T, Jedd G. Import oligomers induce positive feedback to promote peroxisome differentiation and control organelle abundance. Dev Cell. 2011; 21:457–468. Elucidates a cellular mechanism for generating two distinct subpopulations of peroxisomes in the same cell. [PubMed: 21920312]
5. Till A, Lakhani R, Burnett SF, Subramani S. Pexophagy: the selective degradation of peroxisomes. Int J Cell Biol. 2012; 2012:512721. [PubMed: 22536249]
6. Fagarasanu A, Mast FD, Knoblach B, Rachubinski RA. Molecular mechanisms of organelle inheritance: lessons from peroxisomes in yeast. Nature Rev Mol Cell Biol. 2010; 11:644–654. [PubMed: 20717147]
7. De Duve C, Baudhuin P. Peroxisomes (microbodies and related particles). Physiol Rev. 1966; 46:323–357. [PubMed: 5325972]
8. Wanders RJ. Peroxisomes, lipid metabolism, and peroxisomal disorders. Mol Genet Metab. 2004; 83:16–27. [PubMed: 15464416]
9. Grabenbauer M, Satzler K, Baumgart E, Fahimi HD. Three-dimensional ultrastructural analysis of peroxisomes in HepG2 cells. Absence of peroxisomal reticulum but evidence of close spatial association with the endoplasmic reticulum. Cell Biochem Biophys. 2000; 32:37–49. [PubMed: 11330069]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

10. Schrader M, Fahimi HD. Peroxisomes and oxidative stress. Biochim Biophys Acta. 2006; 1763:1755–1766. [PubMed: 17034877]
11. Hoivik DJ, et al. Fibrates induce hepatic peroxisome and mitochondrial proliferation without overt evidence of cellular proliferation and oxidative stress in cynomolgus monkeys. Carcinogenesis. 2004; 25:1757–1769. [PubMed: 15131011]
12. Ishizuka M, et al. Overexpression of human acyl-CoA thioesterase upregulates peroxisome biogenesis. Exp Cell Res. 2004; 297:127–141. [PubMed: 15194431]
13. Jung S, et al. Global analysis of condition-specific subcellular protein distribution and abundance. Mol Cell Proteom. 2013; 12:1421–1435. A novel large-scale analysis of dynamic changes in protein abundance and subcellular compartmentalization in response to an environmental stimulus.
14. Kiel JA, van der Klei IJ, van den Berg MA, Bovenberg RA, Veenhuis M. Overproduction of a single protein, Pc-Pex11p, results in 2-fold enhanced penicillin production by *Penicillium chrysogenum*. Fungal Genet Biol. 2005; 42:154–164. [PubMed: 15670713]
15. Lizana L, Bauer B, Orwar O. Controlling the rates of biochemical reactions and signaling networks by shape and volume changes. Proc Natl Acad Sci USA. 2008; 105:4099–4104. [PubMed: 18337513]
16. Minton AP. How can biochemical reactions within cells differ from those in test tubes? J Cell Sci. 2006; 119:2863–2869. [PubMed: 16825427]
17. Watters D, et al. Localization of a portion of extranuclear ATM to peroxisomes. J Biol Chem. 1999; 274:34277–34282. [PubMed: 10567403]
18. Jung S, Marelli M, Rachubinski RA, Goodlett DR, Aitchison JD. Dynamic changes in the subcellular distribution of Gpd1p in response to cell stress. J Biol Chem. 2010; 285:6739–6749. Shows conditional redistribution of a protein among the cytosol, peroxiomes and the nucleus, and provides evidence for a mechanism to conditionally activate a peroxisomal targeting signal through phosphorylation. [PubMed: 20026609]
19. Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Sinclair DA. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in *Saccharomyces cerevisiae*. Nature. 2003; 423:181–185. [PubMed: 12736687]
20. Beach A, et al. Integration of peroxisomes into an endomembrane system that governs cellular aging. Front Physiol. 2012; 3:283. [PubMed: 22936916]
21. Mignarri A, et al. Zellweger spectrum disorder with mild phenotype caused by *PEX2* gene mutations. JIMD Rep. 2012; 6:43–46. [PubMed: 23430938]
22. Matsui S, Funahashi M, Honda A, Shimozawa N. Newly identified milder phenotype of peroxisome biogenesis disorder caused by mutated *PEX3* gene. Brain Dev. 2012; 35:842–848. [PubMed: 23245813]
23. Thoms S, Gartner J. First PEX11β patient extends spectrum of peroxisomal biogenesis disorder phenotypes. J Med Genet. 2012; 49:314–316. [PubMed: 22581969]
24. Ebberink MS, et al. A novel defect of peroxisome division due to a homozygous non-sense mutation in the *PEX11*β gene. J Med Genet. 2012; 49:307–313. [PubMed: 22581968]
25. Yakunin E, et al. α-synuclein abnormalities in mouse models of peroxisome biogenesis disorders. J Neurosci Res. 2010; 88:866–876. [PubMed: 19830841]
26. Santos MJ, et al. Peroxisomal proliferation protects from β-amyloid neurodegeneration. J Biol Chem. 2005; 280:41057–41068. [PubMed: 16204253]
27. Mast FD, et al. A *Drosophila* model for the Zellweger spectrum of peroxisome biogenesis disorders. Dis Model Mech. 2011; 4:659–672. [PubMed: 21669930]
28. Kiel JA, Veenhuis M, van der Klei IJ. *PEX* genes in fungal genomes: common, rare or redundant. Traffic. 2006; 7:1291–1303. [PubMed: 16978390]
29. Wang X, et al. Multiple targeting modules on peroxisomal proteins are not redundant: discrete functions of targeting signals within Pmp47 and Pex8p. Mol Biol Cell. 2004; 15:1702–1710. [PubMed: 14742703]
30. Walton PA, Hill PE, Subramani S. Import of stably folded proteins into peroxisomes. Mol Biol Cell. 1995; 6:675–683. Shows that stably folded proteins can be imported into peroxisomes. [PubMed: 7579687]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

31. Antonenkov VD, Sormunen RT, Hiltunen JK. The rat liver peroxisomal membrane forms a permeability barrier for cofactors but not for small metabolites *in vitro*. J Cell Sci. 2004; 117:5633–5642. [PubMed: 15509867]
32. Smith JJ, Aitchison JD. Regulation of peroxisome dynamics. Curr Opin Cell Biol. 2009; 21:119–126. [PubMed: 19188056]
33. Erdmann R, Schliebs W. Peroxisomal matrix protein import: the transient pore model. Nature Rev Mol Cell Biol. 2005; 6:738–742. [PubMed: 16103872]
34. Liu X, Ma C, Subramani S. Recent advances in peroxisomal matrix protein import. Curr Opin Cell Biol. 2012; 24:484–489. [PubMed: 22683191]
35. Platta HW, Hagen S, Erdmann R. The exportomer: the peroxisomal receptor export machinery. Cell Mol Life Sci. 2013; 70:1393–1411. [PubMed: 22983384]
36. Meinecke M, et al. The peroxisomal importomer constitutes a large and highly dynamic pore. Nature Cell Biol. 2010; 12:273–277. Gains mechanistic insight into how oligomeric proteins are translocated into peroxisomes. Membrane-associated import receptor Pex5, with its docking partner Pex14, forms a gated ion-conducting channel, which can be opened to a diameter of 9 nm. [PubMed: 20154681]
37. Ma C, Schumann U, Rayapuram N, Subramani S. The peroxisomal matrix import of Pex8p requires only PTS receptors and Pex14p. Mol Biol Cell. 2009; 20:3680–3689. [PubMed: 19570913]
38. Gouveia AM, Reguenga C, Oliveira ME, Sa-Miranda C, Azevedo JE. Characterization of peroxisomal Pex5p from rat liver. Pex5p in the Pex5p–Pex14p membrane complex is a transmembrane protein. J Biol Chem. 2000; 275:32444–32451. [PubMed: 10889202]
39. Kerssen D, et al. Membrane association of the cycling peroxisome import receptor Pex5p. J Biol Chem. 2006; 281:27003–27015. [PubMed: 16849337]
40. Itoh R, Fujiki Y. Functional domains and dynamic assembly of the peroxin Pex14p, the entry site of matrix proteins. J Biol Chem. 2006; 281:10196–10205. [PubMed: 16459329]
41. Schliebs W, et al. Recombinant human peroxisomal targeting signal receptor PEX5. Structural basis for interaction of PEX5 with PEX14. J Biol Chem. 1999; 274:5666–5673. [PubMed: 10026185]
42. Niederhoff K, et al. Yeast Pex14p possesses two functionally distinct Pex5p and one Pex7p binding sites. J Biol Chem. 2005; 280:35571–35578. [PubMed: 16107331]
43. Subramani S. Hitchhiking fads en route to peroxisomes. J Cell Biol. 2002; 156:415–417. [PubMed: 11827979]
44. Gould SJ, Collins CS. Peroxisomal–protein import: is it really that complex? Nature Rev Mol Cell Biol. 2002; 3:382–389. [PubMed: 11988772]
45. Crookes WJ, Olsen LJ. The effects of chaperones and the influence of protein assembly on peroxisomal protein import. J Biol Chem. 1998; 273:17236–17242. [PubMed: 9642294]
46. Freitas MO, et al. PEX5 protein binds monomeric catalase blocking its tetramerization and releases it upon binding the N-terminal domain of PEX14. J Biol Chem. 2011; 286:40509–40519. [PubMed: 21976670]
47. Leon S, et al. Dynamics of the peroxisomal import cycle of PpPex20p: ubiquitin-dependent localization and regulation. J Cell Biol. 2006; 172:67–78. [PubMed: 16390998]
48. Platta HW, et al. Ubiquitination of the peroxisomal import receptor Pex5p is required for its recycling. J Cell Biol. 2007; 177:197–204. [PubMed: 17452527]
49. Platta HW, et al. Pex2 and pex12 function as protein-ubiquitin ligases in peroxisomal protein import. Mol Cell Biol. 2009; 29:5505–5516. [PubMed: 19687296]
50. Platta HW, Grunau S, Rosenkranz K, Girzalsky W, Erdmann R. Functional role of the AAA peroxins in dislocation of the cycling PTS1 receptor back to the cytosol. Nature Cell Biol. 2005; 7:817–822. Identifies a role for AAA peroxins in the ADP-dependent dislocation of the PTS1-receptor from the peroxisomal membrane. [PubMed: 16007078]
51. Miyata N, Fujiki Y. Shuttling mechanism of peroxisome targeting signal type 1 receptor Pex5: ATP-independent import and ATP-dependent export. Mol Cell Biol. 2005; 25:10822–10832. [PubMed: 16314507]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

52. Birschmann I, et al. Pex15p of *Saccharomyces cerevisiae* provides a molecular basis for recruitment of the AAA peroxin Pex6p to peroxisomal membranes. Mol Biol Cell. 2003; 14:2226–2236. [PubMed: 12808025]
53. Matsumoto N, Tamura S, Fujiki Y. The pathogenic peroxin Pex26p recruits the Pex1p–Pex6p AAA ATPase complexes to peroxisomes. Nature Cell Biol. 2003; 5:454–460. [PubMed: 12717447]
54. Schliebs W, Girzalsky W, Erdmann R. Peroxisomal protein import and ERAD: variations on a common theme. Nature Rev Mol Cell Biol. 2010; 11:885–890. [PubMed: 21081964]
55. Gabaldon T, et al. Origin and evolution of the peroxisomal proteome. Biol Direct. 2006; 1:8. [PubMed: 16556314]
56. Schluter A, et al. The evolutionary origin of peroxisomes: an ER–peroxisome connection. Mol Biol Evol. 2006; 23:838–845. [PubMed: 16452116]
57. Leon S, Subramani S. A conserved cysteine residue of *Pichia pastoris* Pex20p is essential for its recycling from the peroxisome to the cytosol. J Biol Chem. 2007; 282:7424–7430. [PubMed: 17209040]
58. Agrawal G, Joshi S, Subramani S. Cell-free sorting of peroxisomal membrane proteins from the endoplasmic reticulum. Proc Natl Acad Sci USA. 2011; 108:9113–9118. [PubMed: 21576455]
59. Hensel A, et al. Cysteine-dependent ubiquitination of Pex18p is linked to cargo translocation across the peroxisomal membrane. J Biol Chem. 2011; 286:43495–43505. [PubMed: 22021076]
60. Glover JR, Andrews DW, Rachubinski RA. S*accharomyces cerevisiae* peroxisomal thiolase is imported as a dimer. Proc Natl Acad Sci USA. 1994; 91:10541–10545. The first example of endogenous import of an oligomeric protein into peroxisomes. [PubMed: 7937990]
61. Yang X, Purdue PE, Lazarow PB. Eci1p uses a PTS1 to enter peroxisomes: either its own or that of a partner, Dci1p. Eur J Cell Biol. 2001; 80:126–138. [PubMed: 11302517]
62. Thoms S, Debelyy MO, Nau K, Meyer HE, Erdmann R. Lpx1p is a peroxisomal lipase required for normal peroxisome morphology. FEBS J. 2008; 275:504–514. [PubMed: 18199283]
63. Williams C, Bener Aksam E, Gunkel K, Veenhuis M, van der Klei IJ. The relevance of the non-canonical PTS1 of peroxisomal catalase. Biochim Biophys Acta. 2012; 1823:1133–1141. Shows the biological relevance of a weak peroxisomal targeting signal and the importance of oligomeric import into peroxisomes for peroxisome function. [PubMed: 22546606]
64. Smith JJ, et al. Transcriptome profiling to identify genes involved in peroxisome assembly and function. J Cell Biol. 2002; 158:259–271. [PubMed: 12135984]
65. Ma C, Hagstrom D, Polley SG, Subramani S. Redox-regulated cargo binding and release by the peroxisomal targeting signal receptor, Pex5. J Biol Chem. 2013; 288:27220–27231. Gains mechanistic understanding of cargo release by the PTS1 receptor into the peroxisomal matrix. Also identifies a mechanism for regulation of matrix protein import in response to the environment. [PubMed: 23902771]
66. Theodoulou FL, Bernhardt K, Linka N, Baker A. Peroxisome membrane proteins: multiple trafficking routes and multiple functions? Biochem J. 2013; 451:345–352. [PubMed: 23581405]
67. Jones JM, Morrell JC, Gould SJ. PEX19 is a predominantly cytosolic chaperone and import receptor for class 1 peroxisomal membrane proteins. J Cell Biol. 2004; 164:57–67. [PubMed: 14709540]
68. Matsuzaki T, Fujiki Y. The peroxisomal membrane protein import receptor Pex3p is directly transported to peroxisomes by a novel Pex19p- and Pex16p-dependent pathway. J Cell Biol. 2008; 183:1275–1286. [PubMed: 19114594]
69. Fransen M, et al. Potential role for Pex19p in assembly of PTS-receptor docking complexes. J Biol Chem. 2004; 279:12615–12624. [PubMed: 14715663]
70. Thoms S, Harms I, Kalies KU, Gartner J. Peroxisome formation requires the endoplasmic reticulum channel protein Sec61. Traffic. 2012; 13:599–609. [PubMed: 22212716]
71. van der Zand A, Braakman I, Tabak HF. Peroxisomal membrane proteins insert into the endoplasmic reticulum. Mol Biol Cell. 2010; 21:2057–2065. [PubMed: 20427571]
72. Schuldiner M, et al. The GET complex mediates insertion of tail-anchored proteins into the ER membrane. Cell. 2008; 134:634–645. Characterizes the mechanism for insertion of tail-anchored proteins including Pex15 into the ER membrane. [PubMed: 18724936]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

73. Kim PK, Mullen RT, Schumann U, Lippincott-Schwartz J. The origin and maintenance of mammalian peroxisomes involves a *de novo* PEX16-dependent pathway from the ER. J Cell Biol. 2006; 173:521–532. Demonstrates that peroxisomes can arise from the ER in mammalian cells and that this is the dominant pathway of peroxisome formation in wild-type cells. [PubMed: 16717127]
74. Motley AM, Hettema EH. Yeast peroxisomes multiply by growth and division. J Cell Biol. 2007; 178:399–410. A landmark paper that develops an assay to follow the fate of existing peroxisomes in live cells. Uses the assay to gain many important insights into both pathways of peroxisome generation. [PubMed: 17646399]
75. Hoepfner D, Schildknegt D, Braakman I, Philippsen P, Tabak HF. Contribution of the endoplasmic reticulum to peroxisome formation. Cell. 2005; 122:85–95. Demonstrates that peroxisomes are generated from domains of the ER. It also reports the development of an assay for following newly made peroxisomes from the ER in live cells for characterizing *de novo* generation of peroxisomes. [PubMed: 16009135]
76. Voorn-Brouwer T, Kragt A, Tabak HF, Distel B. Peroxisomal membrane proteins are properly targeted to peroxisomes in the absence of COPI- and COPII-mediated vesicular transport. J Cell Sci. 2001; 114:2199–2204. [PubMed: 11493655]
77. South ST, Sacksteder KA, Li X, Liu Y, Gould SJ. Inhibitors of COPI and COPII do not block PEX3-mediated peroxisome synthesis. J Cell Biol. 2000; 149:1345–1360. [PubMed: 10871277]
78. Lam SK, Yoda N, Schekman R. A vesicle carrier that mediates peroxisome protein traffic from the endoplasmic reticulum. Proc Natl Acad Sci USA. 2010; 107:21523–21528. Develops the first cell-free preperoxisomal vesicle-budding reaction from microsomal membranes and shows that the process requires Pex19, cytosol and ATP. [PubMed: 21098289]
79. Smith JJ, et al. Expression and functional profiling reveal distinct gene classes involved in fatty acid metabolism. Mol Syst Biol. 2006; 2:2006.0009. [PubMed: 16738555]
80. Lockshon D, Surface LE, Kerr EO, Kaeberlein M, Kennedy BK. The sensitivity of yeast mutants to oleic acid implicates the peroxisome and other processes in membrane function. Genetics. 2007; 175:77–91. [PubMed: 17151231]
81. Perry RJ, Mast FD, Rachubinski RA. Endoplasmic reticulum-associated secretory proteins Sec20p, Sec39p, and Dsl1p are involved in peroxisome biogenesis. Eukaryot Cell. 2009; 8:830–843. Initiates an important movement towards screening essential genes for roles in peroxisome biogenesis. [PubMed: 19346454]
82. 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. Characterizes an elegant mechanism to maintain biochemical identity of peroxisomes during their *de novo* generation from the ER. [PubMed: 22500805]
83. Tam YY, Fagarasanu A, Fagarasanu M, Rachubinski RA. Pex3p initiates the formation of a preperoxisomal compartment from a subdomain of the endoplasmic reticulum in *Saccharomyces cerevisiae*. J Biol Chem. 2005; 280:34933–34939. [PubMed: 16087670]
84. Pan R, Hu J. The conserved fission complex on peroxisomes and mitochondria. Plant Signal Behav. 2011; 6:870–872. [PubMed: 21617372]
85. Loson OC, Song Z, Chen H, Chan DC. Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fission. Mol Biol Cell. 2013; 24:659–667. [PubMed: 23283981]
86. Nagotu S, Saraya R, Otzen M, Veenhuis M, van der Klei IJ. Peroxisome proliferation in *Hansenula polymorpha* requires Dnm1p which mediates fission but not *de novo* formation. Biochim Biophys Acta. 2008; 1783:760–769. [PubMed: 18060881]
87. 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. [PubMed: 12618434]
88. Koch J, Brocard C. PEX11 proteins attract Mff and human Fis1 to coordinate peroxisomal fission. J Cell Sci. 2012; 125:3813–3826. [PubMed: 22595523]
89. Schrader M, Bonekamp NA, Islinger M. Fission and proliferation of peroxisomes. Biochim Biophys Acta. 2012; 1822:1343–1357. [PubMed: 22240198]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

90. Opalinski L, Kiel JA, Williams C, Veenhuis M, van der Klei IJ. Membrane curvature during peroxisome fission requires Pex11. EMBO J. 2011; 30:5–16. [PubMed: 21113128]
91. Schrader M, et al. Expression of PEX11β mediates peroxisome proliferation in the absence of extracellular stimuli. J Biol Chem. 1998; 273:29607–29614. [PubMed: 9792670]
92. Koch A, Yoon Y, Bonekamp NA, McNiven MA, Schrader M. A role for Fis1 in both mitochondrial and peroxisomal fission in mammalian cells. Mol Biol Cell. 2005; 16:5077–5086. [PubMed: 16107562]
93. Motley AM, Ward GP, Hettema EH. Dnm1p-dependent peroxisome fission requires Caf4p, Mdv1p and Fis1p. J Cell Sci. 2008; 121:1633–1640. [PubMed: 18445678]
94. Knoblach B, Rachubinski RA. Phosphorylation-dependent activation of peroxisome proliferator protein PEX11 controls peroxisome abundance. J Biol Chem. 2010; 285:6670–6680. [PubMed: 20028986]
95. Joshi S, Agrawal G, Subramani S. Phosphorylation-dependent Pex11p and Fis1p interaction regulates peroxisome division. Mol Biol Cell. 2012; 23:1307–1315. [PubMed: 22337771]
96. 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. [PubMed: 8858168]
97. Levak-Frank S, et al. Muscle-specific overexpression of lipoprotein lipase causes a severe myopathy characterized by proliferation of mitochondria and peroxisomes in transgenic mice. J Clin Invest. 1995; 96:976–986. [PubMed: 7635990]
98. Epstein CB, et al. Genome-wide responses to mitochondrial dysfunction. Mol Biol Cell. 2001; 12:297–308. Shows that peroxisome biogenesis is dramatically induced by respiratory deficiency and identifies the cellular coordination of peroxisome biogenesis with mitochondrial function. [PubMed: 11179416]
99. Butow RA, Avadhani NG. Mitochondrial signaling: the retrograde response. Mol Cell. 2004; 14:1–15. [PubMed: 15068799]
100. Titorenko VI, Chan H, Rachubinski RA. Fusion of small peroxisomal vesicles *in vitro* reconstructs an early step in the *in vivo* multistep peroxisome assembly pathway of *Yarrowia lipolytica*. J Cell Biol. 2000; 148:29–44. [PubMed: 10629216]
101. Titorenko VI, Rachubinski RA. Peroxisomal membrane fusion requires two AAA family ATPases, Pex1p and Pex6p. J Cell Biol. 2000; 150:881–886. Identifies and characterizes the mechanism of vesicle fusion in peroxisome biogenesis. [PubMed: 10953011]
102. Doherty GJ, McMahon HT. Mechanisms of endocytosis. Annu Rev Biochem. 2009; 78:857–902. [PubMed: 19317650]
103. Nickel W, Rabouille C. Mechanisms of regulated unconventional protein secretion. Nature Rev Mol Cell Biol. 2009; 10:148–155. [PubMed: 19122676]
104. Saftig P, Klumperman J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nature Rev Mol Cell Biol. 2009; 10:623–635. [PubMed: 19672277]
105. Yan M, Rayapuram N, Subramani S. The control of peroxisome number and size during division and proliferation. Curr Opin Cell Biol. 2005; 17:376–383. [PubMed: 15978793]
106. Lazarow PB. Peroxisome biogenesis: advances and conundrums. Curr Opin Cell Biol. 2003; 15:489–497. [PubMed: 12892791]
107. Saraya R, Krikken AM, Veenhuis M, van der Klei IJ. Peroxisome reintroduction in *Hansenula polymorpha* requires Pex25 and Rho1. J Cell Biol. 2011; 193:885–900. [PubMed: 21606207]
108. Waterham HR, Ebberink MS. Genetics and molecular basis of human peroxisome biogenesis disorders. Biochim Biophys Acta. 2012; 1822:1430–1441. [PubMed: 22871920]
109. Fagarasanu A, Fagarasanu M, Eitzen GA, Aitchison JD, Rachubinski RA. The peroxisomal membrane protein Inp2p is the peroxisome-specific receptor for the myosin V motor Myo2p of *Saccharomyces cerevisiae*. Dev Cell. 2006; 10:587–600. [PubMed: 16678774]
110. Fagarasanu M, Fagarasanu A, Tam YY, Aitchison JD, Rachubinski RA. Inp1p is a peroxisomal membrane protein required for peroxisome inheritance in *Saccharomyces cerevisiae*. J Cell Biol. 2005; 169:765–775. Describes the first peroxisomal protein directly implicated in peroxisome inheritance. [PubMed: 15928207]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

111. Reggiori F, Monastyrska I, Shintani T, Klionsky DJ. The actin cytoskeleton is required for selective types of autophagy, but not nonspecific autophagy, in the yeast *Saccharomyces cerevisiae*. Mol Biol Cell. 2005; 16:5843–5856. [PubMed: 16221887]
112. Bharti P, et al. PEX14 is required for microtubule-based peroxisome motility in human cells. J Cell Sci. 2011; 124:1759–1768. [PubMed: 21525035]
113. Stelter P, et al. Molecular basis for the functional interaction of dynein light chain with the nuclear-pore complex. Nature Cell Biol. 2007; 9:788–796. [PubMed: 17546040]
114. Zutphen T, Veenhuis M, van der Klei IJ. Pex14 is the sole component of the peroxisomal translocon that is required for pexophagy. Autophagy. 2008; 4:63–66. [PubMed: 17921697]
115. Cogliati S, et al. Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. Cell. 2013; 155:160–171. [PubMed: 24055366]
116. Koerkamp MG, et al. Dissection of transient oxidative stress response in *Saccharomyces cerevisiae* by using DNA microarrays. Mol Biol Cell. 2002; 13:2783–2794. [PubMed: 12181346]
117. Smith JJ, et al. Transcriptional responses to fatty acid are coordinated by combinatorial control. Mol Syst Biol. 2007; 3:115. [PubMed: 17551510]
118. 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. [PubMed: 10787422]
119. Poll-The BT, 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. [PubMed: 2894756]
120. Chang CC, et al. Metabolic control of peroxisome abundance. J Cell Sci. 1999; 112:1579–1590. [PubMed: 10212151]
121. Saleem RA, et al. Integrated phosphoproteomics analysis of a signaling network governing nutrient response and peroxisome induction. Mol Cell Proteom. 2010; 9:2076–2088.
122. Guo T, et al. A signal from inside the peroxisome initiates its division by promoting the remodeling of the peroxisomal membrane. J Cell Biol. 2007; 177:289–303. [PubMed: 17438077]
123. Knoblach B, et al. An ER–peroxisome tether exerts peroxisome population control in yeast. EMBO J. 2013; 32:2439–2453. [PubMed: 23900285]
124. David C, et al. A combined approach of quantitative interaction proteomics and live-cell imaging reveals a regulatory role for endoplasmic reticulum (ER) reticulon homology proteins in peroxisome biogenesis. Mol Cell Proteom. 2013; 12:2408–2425.
125. Motley AM, Nuttall JM, Hettema EH. Pex3-anchored Atg36 tags peroxisomes for degradation in *Saccharomyces cerevisiae*. EMBO J. 2012; 31:2852–2868. [PubMed: 22643220]
126. Bellu AR, Salomons FA, Kiel JA, Veenhuis M, Van Der Klei IJ. Removal of Pex3p is an important initial stage in selective peroxisome degradation in *Hansenula polymorpha*. J Biol Chem. 2002; 277:42875–42880. [PubMed: 12221086]
127. Farre JC, Manjithaya R, Mathewson RD, Subramani S. PpAtg30 tags peroxisomes for turnover by selective autophagy. Dev Cell. 2008; 14:365–376. [PubMed: 18331717]
128. van Zutphen T, Veenhuis M, van der Klei IJ. Damaged peroxisomes are subject to rapid autophagic degradation in the yeast *Hansenula polymorpha*. Autophagy. 2011; 7:863–872. [PubMed: 21490428]
129. Munck JM, Motley AM, Nuttall JM, Hettema EH. A dual function for Pex3p in peroxisome formation and inheritance. J Cell Biol. 2009; 187:463–471. [PubMed: 19948495]
130. Chang J, et al. Pex3 peroxisome biogenesis proteins function in peroxisome inheritance as class V myosin receptors. J Cell Biol. 2009; 187:233–246. [PubMed: 19822674]
131. Huber A, Koch J, Kragler F, Brocard C, Hartig A. A subtle interplay between three Pex11 proteins shapes *de novo* formation and fission of peroxisomes. Traffic. 2012; 13:157–167. [PubMed: 21951626]
132. Krikken AM, Veenhuis M, van der Klei IJ. *Hansenula polymorpha pex11* cells are affected in peroxisome retention. FEBS J. 2009; 276:1429–1439. [PubMed: 19187239]
133. Otzen M, et al. Pex19p contributes to peroxisome inheritance in the association of peroxisomes to Myo2p. Traffic. 2012; 13:947–959. [PubMed: 22486971]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

134. Bellu AR, Komori M, van der Klei IJ, Kiel JA, Veenhuis M. Peroxisome biogenesis and selective degradation converge at Pex14p. J Biol Chem. 2001; 276:44570–44574. [PubMed: 11564741]

135. Lazarow PB, Fujiki Y. Biogenesis of peroxisomes. Annu Rev Cell Biol. 1985; 1:489–530. [PubMed: 3916321]

136. Yamamoto K, Fahimi HD. Three-dimensional reconstruction of a peroxisomal reticulum in regenerating rat liver: evidence of interconnections between heterogeneous segments. J Cell Biol. 1987; 105:713–722. [PubMed: 2887576]

137. Schrader M, Wodopia R, Fahimi HD. Induction of tubular peroxisomes by UV irradiation and reactive oxygen species in HepG2 cells. J Histochem Cytochem. 1999; 47:1141–1148. [PubMed: 10449535]

138. Islinger M, Abdolzade-Bavil A, Liebler S, Weber G, Volkl A. Assessing heterogeneity of peroxisomes: isolation of two subpopulations from rat liver. Methods Mol Biol. 2012; 909:83–96. [PubMed: 22903710]

139. Manivannan S, de Boer R, Veenhuis M, van der Klei IJ. Lumenal peroxisomal protein aggregates are removed by concerted fission and autophagy events. Autophagy. 2013; 9:1044–1056. Identifies a cellular process generating two distinct subpopulations of peroxisomes in the same cell by a mechanism involving protein aggregation and asymmetric fission. [PubMed: 23614977]

140. Liu F, et al. Making two organelles from one: Woronin body biogenesis by peroxisomal protein sorting. J Cell Biol. 2008; 180:325–339. [PubMed: 18227279]

141. Nishimura M, Hayashi M, Kato A, Yamaguchi K, Mano S. Functional transformation of microbodies in higher plant cells. Cell Struct Funct. 1996; 21:387–393. [PubMed: 9118245]

142. Moyersoen J, Choe J, Fan E, Hol WG, Michels PA. Biogenesis of peroxisomes and glycosomes: trypanosomatid glycosome assembly is a promising new drug target. FEMS Microbiol Rev. 2004; 28:603–643. [PubMed: 15539076]

143. Saleem RA, et al. Genome-wide analysis of signaling networks regulating fatty acid-induced gene expression and organelle biogenesis. J Cell Biol. 2008; 181:281–292. [PubMed: 18426976]

144. Butow RA. Cellular responses to mitochondrial dysfunction: it's not always downhill. Cell Death Differ. 2002; 9:1043–1045. [PubMed: 12232791]

145. Danziger, SA., et al. Molecular mechanisms of system responses to novel stimuli are predictable from public datasets. Nucleic Acids Res. 2013. http://dx.doi.org/10.1093/nar/gkt938

146. Zeigerer A, et al. Rab5 is necessary for the biogenesis of the endolysosomal system *in vivo*. Nature. 2012; 485:465–470. [PubMed: 22622570]

147. Binder B, Goede A, Berndt N, Holzhutter HG. A conceptual mathematical model of the dynamic self-organisation of distinct cellular organelles. PLoS ONE. 2009; 4:e8295. [PubMed: 20041124]

148. Nagan N, Zoeller RA. Plasmalogens: biosynthesis and functions. Prog Lipid Res. 2001; 40:199–229. [PubMed: 11275267]

149. Diano S, et al. Peroxisome proliferation-associated control of reactive oxygen species sets melanocortin tone and feeding in diet-induced obesity. Nature Med. 2011; 17:1121–1127. [PubMed: 21873987]

150. Dixit E, et al. Peroxisomes are signaling platforms for antiviral innate immunity. Cell. 2010; 141:668–681. Identifies a role for peroxisomes in antiviral signal transduction in macrophages. [PubMed: 20451243]

151. Smith JJ, et al. Environment-responsive transcription factors bind subtelomeric elements and regulate gene silencing. Mol Syst Biol. 2011; 7:455. [PubMed: 21206489]

152. Smith JJ, Saleem RA, Aitchison JD. Statistical analysis of dynamic transcriptional regulatory network structure. Methods Mol Biol. 2011; 781:337–352. [PubMed: 21877289]

153. Ratushny AV, et al. Control of transcriptional variability by overlapping feed-forward regulatory motifs. Biophys J. 2008; 95:3715–3723. [PubMed: 18621837]

154. Ratushny AV, Saleem RA, Sitko K, Ramsey SA, Aitchison JD. Asymmetric positive feedback loops reliably control biological responses. Mol Syst Biol. 2012; 8:577. [PubMed: 22531117]

155. Ratushny AV, Ramsey SA, Aitchison JD. Mathematical modeling of biomolecular network dynamics. Methods Mol Biol. 2011; 781:415–433. [PubMed: 21877294]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

156. Ratushny AV, Shmulevich I, Aitchison JD. Trade-off between responsiveness and noise suppression in biomolecular system responses to environmental cues. PLoS Comput Biol. 2011; 7:e1002091. [PubMed: 21738459]

157. Ramsey SA, et al. Dual feedback loops in the *GAL* regulon suppress cellular heterogeneity in yeast. Nature Genet. 2006; 38:1082–1087. [PubMed: 16936734]

158. van Zutphen T, van der Klei IJ. Quantitative analysis of organelle abundance, morphology and dynamics. Curr Opin Biotechnol. 2011; 22:127–132. [PubMed: 21106361]

159. Bassett D, et al. Detecting patterns of protein distribution and gene expression *in silico*. Proc Natl Acad Sci USA. 2005; 102:516. [PubMed: 15696628]

160. Kal AJ, et al. *In silicio* search for genes encoding peroxisomal proteins in *Saccharomyces cerevisiae*. Cell Biochem Biophys. 2000; 32:1–8. [PubMed: 11330035]

161. Marelli M, 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. A global analysis of the peroxisomal proteome. Uses an isotope-coded affinity tagging approach to calculate the relative enrichment of proteins with peroxisomal membranes and to eliminate false positives. [PubMed: 15596542]

162. Yi EC, et al. Approaching complete peroxisome characterization by gas-phase fractionation. Electrophoresis. 2002; 23:3205–3216. [PubMed: 12298092]

163. Schafer H, Nau K, Sickmann A, Erdmann R, Meyer HE. Identification of peroxisomal membrane proteins of *Saccharomyces cerevisiae* by mass spectrometry. Electrophoresis. 2001; 22:2955–2968. [PubMed: 11565790]

164. Saleem RA, et al. Genome-wide analysis of effectors of peroxisome biogenesis. PLoS ONE. 2010; 5:e11953. [PubMed: 20694151]

165. Wolinski H, et al. Imaging-based live cell yeast screen identifies novel factors involved in peroxisome assembly. J Proteome Res. 2009; 8:20–27. [PubMed: 19118449]

166. Knijnenburg TA, et al. A regression model approach to enable cell morphology correction in high-throughput flow cytometry. Mol Syst Biol. 2011; 7:531. [PubMed: 21952134]

167. Halbach A, et al. Targeting of the tail-anchored peroxisomal membrane proteins PEX26 and PEX15 occurs through C-terminal PEX19-binding sites. J Cell Sci. 2006; 119:2508–2517. [PubMed: 16763195]

168. Managadze D, et al. Identification of PEX33, a novel component of the peroxisomal docking complex in the filamentous fungus *Neurospora crassa*. Eur J Cell Biol. 2010; 89:955–964. [PubMed: 20728240]

169. Kuravi K, et al. Dynamin-related proteins Vps1p and Dnm1p control peroxisome abundance in *Saccharomyces cerevisiae*. J Cell Sci. 2006; 119:3994–4001. [PubMed: 16968746]

170. Jeynov B, Lay D, Schmidt F, Tahirovic S, Just WW. Phosphoinositide synthesis and degradation in isolated rat liver peroxisomes. FEBS Lett. 2006; 580:5917–5924. [PubMed: 17045591]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

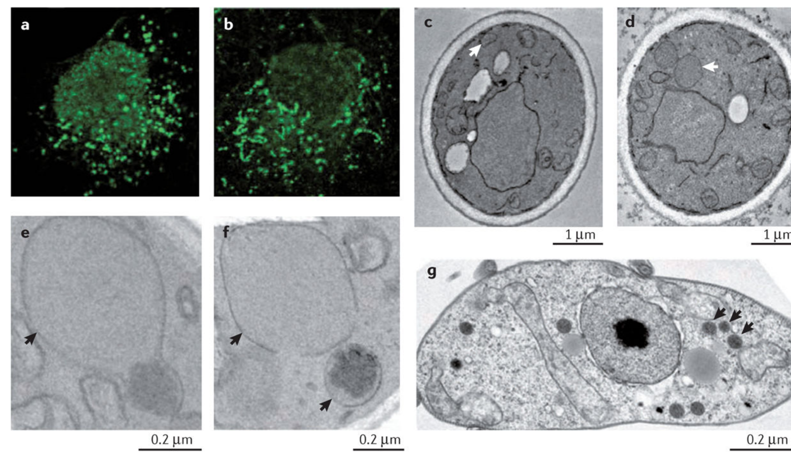


Figure 1. Peroxisome dynamics

a,**b** | Immunofluorescence microscopy images of COS-7 cells (African green monkey kidney cells) stained with an antibody against peroxisomal targeting signal 1 (PTS1) (anti-SKL). Different cells in the same culture have different peroxisome morphologies; one cell has spherical peroxisomes (part **a**), whereas the other cell has both elongated peroxisomes and rows of spherical peroxisomes that are likely to be derived from fission of tubular peroxisomes (part **b**). **c**,**d** | Electron micrographs of the yeast *Saccharomyces cerevisiae* in the absence (part **c**) and presence (part **d**) of fatty acids showing proliferation of peroxisomes (indicated by arrows) in response to the stimulus. **e**,**f** | Electron micrographs showing peroxisomes in the yeast *Hansenula polymorpha* during the development of aggregate-containing peroxisomes (indicated by arrows). The strain *agt1*-Cat^{mut} has blocked autophagic degradation and expresses a mutant version of catalase that form the aggregates, which are sequestered (part **e**) and separated from the mother organelle by asymmetric peroxisome fission (part **f**). **g** | Electron micrograph of *Trypansoma brucei* showing a class of peroxisomes called glycosomes (indicated by arrows). The images in parts **e** and **f** are reproduced, with permission, from REF. 139 © (2013) Landes Bioscience. The image in part **g** courtesy of Sanjiban Banerjee and Marilyn Parsons, Seattle Biomedical Research Institute, Seattle, Washington, USA.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

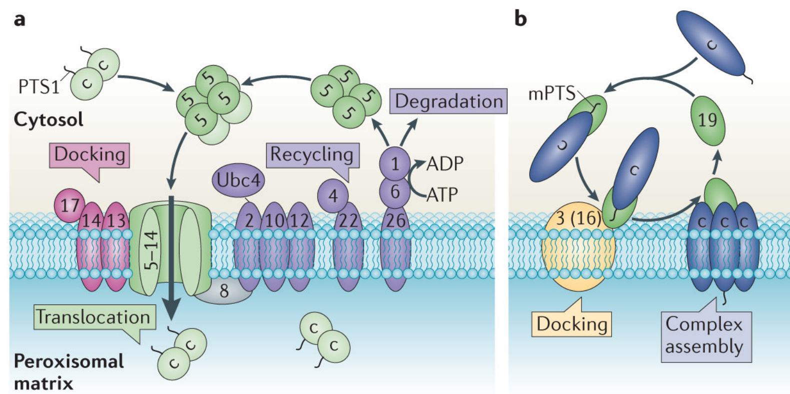


Figure 2. Direct targeting of proteins to peroxisomes

a | Import of matrix proteins into peroxisomes. Import is shown for a peroxisomal targeting signal 1 (PTS1)-containing cargo (c) imported as a multimer into peroxisomes in yeast. The PTS1 receptor Pex5 interacts with the PTS1 of cargo in the cytoplasm, docks at the docking complex (pink) and is integrated into the membrane to form the transport channel with Pex14. Cargo is released from Pex5 and imported into peroxisomes, and Pex5 is ubiquitylated (not shown) and extracted from the membrane in an ATP-dependent manner by the exportomer[35] (shown in purple). Monoubiquitylation of Pex5 by ubiquitin-conjugating and ligase enzymes, Pex4 and Pex12, respectively, enables Pex5 recycling back to the cytosol for another round of import, whereas polyubiquitylation (by the ubiquitin-conjugating and ligase enzymes, Ubc4 and Pex2, respectively) directs Pex5 to the proteasome for degradation. Pex5 is shown as a tetramer in the cytosol reflecting the mechanistic details of cargo binding and release in *Pichia pastoris*[65]; Pex5 functions as a tetramer in the cytosol, and as a dimer or heterooligomer (with Pex8) in the peroxisomal membrane. These states seem to be redox-regulated, which promotes cargo binding in the cytoplasm and release in the reducing environment of the peroxisomal lumen. Note that there may be different oligomeric states of PEX5 and functions of each in different organisms[65]. Although most components of the import machinery are evolutionarily conserved, there are notable exceptions, including the existence of functional homologues of PEX26 in *Saccharomyces cerevisiae* (Pex15)[52,167] and PEX17 in *Neurospora crassa* (Pex33)[168]. In addition, several peroxins, including Pex22, Pex8, Pex4 and Pex17, have not been identified in higher eukaryotes (TABLE 2). **b** | Import of membrane proteins into peroxisomes. In the cytoplasm, the peroxisomal membrane protein (PMP) is targeted directly to peroxisomes through the interaction of its membrane PTS (mPTS) with the shuttling receptor Pex19 in the cytoplasm, and through docking of this complex to Pex3 (or PEX16 in mammalian cells) at the peroxisomal membrane. Pex19 then mediates the assembly of PMPs into complexes (shown in blue). PMPs can also be targeted to peroxisomes by insertion into the endoplasmic reticulum (ER) membrane followed by vesicular transport to peroxisomes (FIG. 3).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

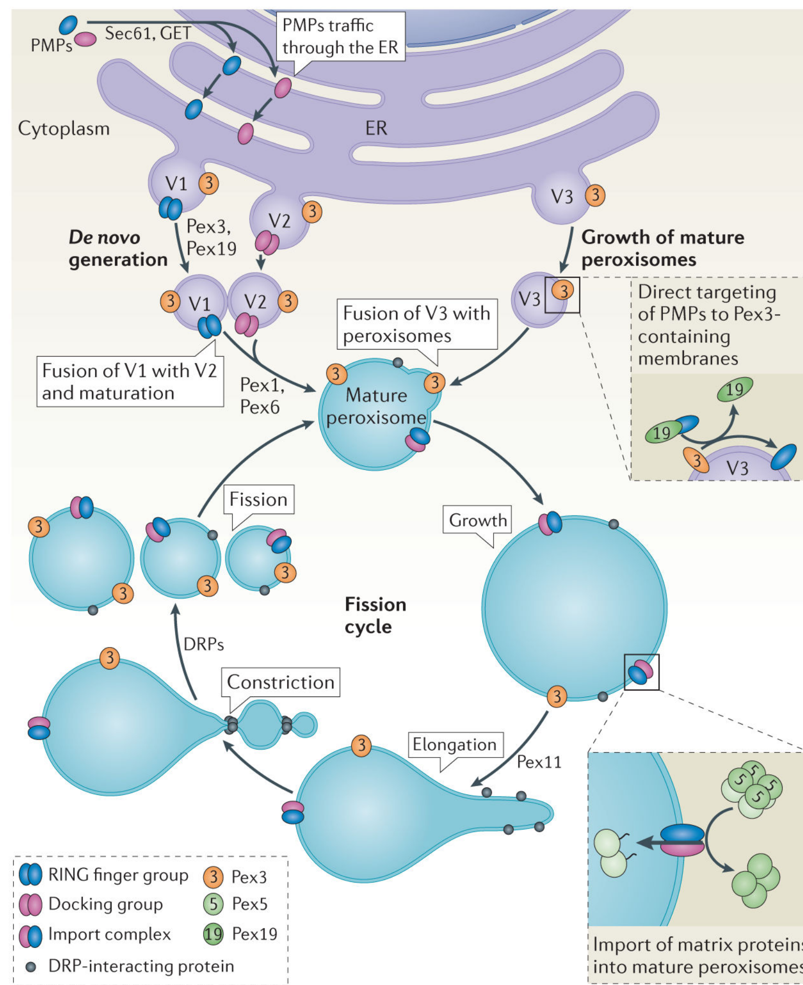


Figure 3. Peroxisomes can form through two pathways

Details of peroxisome formation by *de novo* generation, and by growth and division are shown for yeast. Peroxisomes are formed *de novo* from the endoplasmic reticulum (ER) through budding and pair-wise heterotypic fusion of two vesicle types, V1 and V2 (left). This mechanism separates RING finger and docking components of the import complex into different vesicles, which are not import competent until after fusion and assembly of a complete and functional import complex. Peroxin 1 (Pex1) and Pex6 are found in separate preperoxisomal vesicles and are necessary for the heterotypic fusion of V1 and V2 vesicles and the formation of mature, import-competent peroxisomes. Mature peroxisomes can multiply by growth, with proteins and membranes from the ER (via V3 vesicles; right), and fission, mediated by Pex11. Although all preperoxisomal vesicles characterized contain Pex3 (which is required for egression from the ER), V3 vesicles are distinct from V1 and V2 vesicles because they can fuse with mature peroxisomes. A fission cycle begins with membrane remodelling and elongation mediated by Pex11. The elongated extension grows and acquires DRP (dynamin-related protein)-interacting proteins (including the fission protein Fis1 (REFS 88,93,95)). The membrane becomes constricted by an unknown mechanism, and DRPs (Dnm1 and vacuolar sorting protein 1 (Vsp1) in yeast[169] or dynamin-like protein DLP1 in mammalian cells), which are recruited from the cytosol by DRP-

interacting proteins, facilitate membrane fission to generate new peroxisomes. Note that for simplicity, not all peroxins are shown.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 1

Functions of peroxins in peroxisome biogenesis

Peroxin	Functional categories
Targeting of matrix proteins	
PEX7	PTS2 cargo, shuttling receptor
PEX20	PTS2 cargo, co-receptor
PEX21[*] (PEX18 paralogue)	PTS2 cargo, co-receptor
PEX18[*] (PEX21 paralogue)	PTS2 cargo, co-receptor,
PEX5	PTS1 and PTS2 cargo, shuttling receptor
Matrix protein import machinery	
PEX5[‡], PEX14[‡]	Cargo-translocating channel
PEX13, PEX14[‡], PEX17, PEX33	Receptor docking complex
PEX8	Docking and export complex conjugation, importomer assembly
PEX4	Receptor export (ubiquitylation), ubiquitin conjugating enzyme
PEX22	Receptor export (ubiquitylation), Pex4 anchor
PEX2, PEX10, PEX12	Receptor export (ubiquitylation), form the RING finger complex
PEX1[‡], PEX6[‡]	Receptor export (recycling), AAA-type ATPase
PEX15, PEX26	Receptor export (recycling), membrane receptors for Pex1 and Pex6
Direct targeting of PMPs	
PEX3	Receptor docking
PEX19	Soluble chaperone and receptor
Formation of peroxisomal membrane from the ER	
PEX3[*,‡], PEX19[‡]	Form a complex required for the *de novo* generation of peroxisomes
PEX25[‡] (PEX11 family member)	Required for the *de novo* generation of peroxisomes, recruits Rho1
PEX16	Recruits PMPs in the ER
PEX1[‡], PEX6[‡]	Mediate fusion of preperoxisomal vesicles in the *de novo* formation of peroxisomes
PEX23[*,‡], PEX30[*,‡]	Regulate the *de novo* generation of peroxisomes
Fission	
PEX11[*,‡] (PEX11 family member)	Membrane elongation, recruits the fission machinery
PEX25[*,‡] (PEX11 family member)	Membrane elongation and remodelling
PEX27[*] (PEX11 family member)	Negatively affects fission
PEX34	Positive regulator of fission
Regulation of peroxisome biogenesis	
PEX24, PEX28, PEX29, PEX23[*,‡], PEX30[*,‡], PEX32[*]	Form a complex with reticulon homology domain- containing proteins and establish peroxisome contact sites at ER subdomains, contain dysferlin domains
PEX31[*]	Contains a dysferlin domain

PEX, peroxin; PTS, peroxisomal targeting signal; ER, endoplasmic reticulum; PMPs, peroxisomal membrane proteins.

*These peroxins have multiple isoforms or family members within the same organism.

‡These peroxins are multifunctional. PEX9 is not listed because the open reading frame was wrongly identified and seems to be PEX26 (REF. 28).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 2

PEX genes in various model organisms

Gene	Sc	Yl	Hp	Nc	Dm	Mm	Hs	PBD
PEX1	✓	✓	✓	✓	✓	✓	✓	IRD, NALD, ZSS
PEX2	✓	✓	✓	✓	✓	✓	✓	ZS, Mild ZSS*
PEX3	✓ (3,3B)	✓	✓	✓	✓	✓	✓	ZS, Mild IRD‡
PEX4	✓	✓	✓	✓				
PEX5	✓ (5,5C§)	✓	✓	✓	✓	✓ (5,5l)	✓ (S,L)	NALD, ZSS
PEX6	✓	✓	✓	✓	✓	✓	✓	NALD, ZSS
PEX7	✓	✓	✓	✓	✓	✓	✓	RCDP
PEX8	✓	✓	✓	✓				
PEX10	✓	✓	✓	✓	✓	✓	✓	NALD, ZSS
PEX11	✓	✓	✓ (11,11C)	✓ (A,B,C)	(A,B,C)	✓ (α,β)	✓ (α,β,γ)	Mild ZSS (β)//
PEX12	✓	✓	✓	✓	✓	✓	✓	IRD, NALD, ZSS
PEX13	✓	✓	✓	✓	✓	✓	✓	NALD, ZSS
PEX14	✓	✓	✓	✓	✓	✓	✓	ZS
PEX15	✓							
PEX16		✓		✓	✓	✓	✓	ZSS
PEX17	✓	✓	✓					
PEX18	✓							
PEX19	✓	✓	✓	✓	✓	✓	✓	ZS
PEX20		✓	✓	✓	✓			
PEX21	✓							
PEX22	✓	✓	✓	✓				
PEX23, PEX30	✓ (30,23l)	✓ (23,23l)	✓ (23,23l)	✓ (23,23l)	✓ (23)		✓ (23)	
PEX24, PEX28	✓ (28)	✓ (24)	✓ (24)	✓ (24)				
PEX25	✓	✓¶	✓					
PEX26		✓	✓	✓		✓	✓	ZSS, NALD, IRD
PEX27	✓							

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Gene	Sc	Yl	Hp	Nc	Dm	Mm	Hs	PBD
PEX29	✓	✓	✓					
PEX31	✓							
PEX32	✓		✓					
PEX33				✓				
PEX34	✓							

Dm, Drosophila melanogaster; Hp, Hansenula polymorpha, Hs, Homo sapiens; IRD, infantile refsum disease; Mm, Mus musculus; NALD, neonatal adrenoleukodystrophy; Nc, *Neurospora crassa; PEX*, peroxin; PBD, peroxisome biogenesis disorder; RCDP, rhizomelic chondrodysplasia punctata; *Sc*, *Saccharomyces cerevisiae*; Yl, *Yarrowia lipolytica*; ZS, Zellweger syndrome; ZSS, ZS spectrum.

*Slow progression (diagnosed at 51 years)[21].

‡Slow progression (diagnosed >30 years) with late-onset leukodystrophy[22].

§This open reading frame, *YMR018W*[28], has a predicted role in peroxisome biogenesis or function as indicated by transcriptome profiling[64].

//Slow progression (diagnosed at 26 years) with ZSS-atypical symptoms, including mild intellectual disability[23,24].

¶The encoded protein has weak similarity to Pex11 and Pex25 (REF. 28). Fungal annotations are primarily taken from REF. 28 and *D. melanogaster* annotations are taken from REF. 27. Human *PEX23* annotation is taken from REF. 170. *Y. lipolytica PEX9* is shown as *PEX26*, and *S. cerevisiae PEX30* and *PEX28* are grouped with the presumed orthologues, *PEX23* and *PEX24*, respectively[28]. Protein isoforms are shown in parentheses, including genes that are annotated 23l encoding Pex23-like proteins[28].