Peroxisomes: simple in function but complex in maintenance

Henk F. Tabak, Ineke Braakman and Ben Distel

Peroxisomes compartmentalize part of the anabolic and catabolic pathways and reactions of the cell. Dysfunction of a single peroxisomal enzyme or loss of the whole peroxisomal compartment causes sporadic, but serious, human diseases. Genetic studies in various yeasts have identified PEX genes, which are required for the maintenance of complete peroxisomes. Mutations in PEX genes have proved to be the molecular cause of several human diseases, particularly those involving loss of organelles. Peroxisomes have several properties that distinguish them from other organelles, including the import of folded proteins from the cytosol by an unknown mechanism. By discussing recent highlights from the field of peroxisome research, we aim to share with the general readership our excitement as well as the many mysteries still surrounding peroxisome function and maintenance.

Peroxisomes were discovered as biochemical entities by De Duve's group. They were identified as small sedimentable particles containing marker enzymes that distinguished them from other known organelles[1]. The presence of hydrogen-peroxide-producing oxidases and catalase inspired the name peroxisomes and focused attention on their role in oxidative metabolic transactions. Despite having appeared in the literature for almost half a century, peroxisomes have yet to find their rightful place in biochemical and cell-biological textbooks. There are a number of reasons for this. First, we do not understand why they exist in the first place. Are they remnants of early symbionts that have completely lost their DNA? Attempts to find homologies among evolutionarily conserved proteins do not overtly support a bacterial origin. The remarkable variability in enzyme content in different species is also not consistent with this concept. Second, there are no good reasons for peroxisomal metabolism to be compartmentalized and separated from the remainder of the cell. Many reactive oxygen species arising within peroxisomes are membrane permeant[2]. Indeed, a particular *Hansenula polymorpha* mutant can grow without peroxisomes, using the peroxisomal enzymes located in the cytosol[3]. Finally, the variability of protein content in different species hindered early recognition of microbodies as one well-defined group of cellular organelles. Microbodies comprise peroxisomes, glycosomes, glyoxysomes and possibly (some) hydrogenosomes. The recent recognition that the proper functioning of peroxisomes is important for human health and the observation that central processes such as import of proteins into peroxisomes are not simply reiterations of established principles are contributing to a renewed interest in the organelle. As is often the case in a rapidly developing field, contradictory experimental findings and differences of opinion abound. In this review, we avoid some of the minor controversial issues that are of particular interest only to the aficionados in the field. Instead, we address the general readers – to convince them that peroxisomes have entered the major league of cell biology. For in-depth discussions of particular issues, readers are referred to the review articles listed in Box 1.

Peroxisome metabolism

Isolated peroxisomes are permeable to small molecules such as sucrose. During isolation, they often lose proteins that are normally confined to the peroxisomal matrix. This loss of peroxisomal content was initially taken as evidence for the permeability of the peroxisomal membrane *in vivo*, but is now known to be an isolation artifact. In living cells, peroxisomes are sealed vesicles surrounded by a single membrane. Dedicated membrane proteins are required to allow communication across the peroxisomal membrane[3,4]. The first transporter molecules to be identified were the ABC half-transporters Pat1p and Pat2p of *Saccharomyces cerevisiae*. These proteins are homologous to the human peroxisomal ABC transporter, which, when mutated, is responsible for X-linked adrenoleukodystrophy[5,6]. In-depth analysis of the function of Pat1p and Pat2p in yeast suggests that they are important for the translocation of long-chain acyl-CoA esters across the peroxisomal membrane for subsequent β-oxidation within the peroxisomal matrix[7,8]. In *S. cerevisiae*, fatty acid oxidation takes place only in peroxisomes[9]. In mammalian cells, shortened fatty acids are exported from peroxisomes to mitochondria for complete degradation[10]. The absence of mitochondrial isoenzymes for β-oxidation means that acetyl-CoA and NADH are produced in yeast peroxisomes (Fig. 1). This makes *S. cerevisiae* an ideal model organism for peroxisome studies. NADH is reoxidized by peroxisomal malate dehydrogenase, and malate is formed[4]. Malate is likely to be exported to the cytosol for oxidation into oxaloacetate by cytosolic malate dehydrogenase. Sustained β-oxidation is thus dependent on a malate–oxaloacetate shuttle across the membrane or on a more complex derivative of this shuttle. Acetyl-CoA in yeast can be assimilated to succinate by the glyoxylate cycle; some

The authors are in the Dept of Biochemistry, Academic Medical Center, University of Amsterdam, 1105 AZ, Amsterdam, The Netherlands. E-mail: h.f.tabak@amc.uva.nl

0962-8924/99/$ – see front matter

PII: S0962-8924(99)01650-5

BOX 1 – FURTHER READING

- **History and first characterization of peroxisomes as biochemical entities**
  - De Duve, C. and Bauduin, P. (1966) Peroxisomes (microbodies and related particles). *Physiol. Rev*. 46, 323–357
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- **Peroxisomes multiply by growth and division and import proteins from the cytosol**
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- **Peroxisomal metabolism**
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  - Elgersma, Y. and Tabak, H. F. (1996) Proteins involved in peroxisome biogenesis and functioning. *Biochim. Biophys. Acta* 1286, 269–283
- **Peroxisomal-targeting sequences**
  - Subramani, S. (1993) Protein import into peroxisomes and biogenesis of the organelle. *Annu. Rev. Cell Biol*. 9, 445–478
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  - Subramani, S. (1997) *PEX* genes on the rise. *Nat. Genet*. 15, 331–333
- **Thoughtful remarks on organelle inheritance in general**
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of the enzymes involved are confined to peroxisomes. An alternative route for exit of acetyl-CoA is via conversion to acetylcarnitine by the peroxisomal enzyme carnitine acetyltransferase[11]. Although our knowledge of metabolic pathways is more extensive in mammals, work in yeast allows us to understand how they integrate into the complete metabolic context of the cell.

Degradation of some polyunsaturated fatty acids requires intramolecular relocation of double bonds. The enzymes involved require NADPH to carry out this function. Cytosolic and peroxisomal NADP-dependent isocitrate dehydrogenases might function in a shuttle in a manner similar to the malate dehydrogenases. The goal is different, however – to keep the intraperoxisomal pool of NADP reduced[12–14]. This results in the simultaneous flow of reduction equivalents in opposite directions, which are kept separated by different cofactors (NAD/NADP) and substrates (malate/citrate). Insight into peroxisomal metabolism has led us to postulate an increasing number of integral membrane proteins, such as Pat1p and Pat2p, that mediate the shuttling of various metabolites across the peroxisomal membrane. In this respect, peroxisomes lag behind the mitochondria, for which many different carriers in the inner membrane have already been identified[15].

Peroxisomes constitute a dynamic compartment

The number and volume of peroxisomes are dependent on external conditions (Fig. 2). Feeding of hypolipidaemic drugs to rats enlarges the peroxisomal compartment[16]. These drugs probably act through the nuclear hormone receptor PPAR-α in

conjunction with RXR-α[17]. Natural ligands include long-chain, branched-chain or polyunsaturated fatty acids and eicosanoids that stimulate transcription of genes containing a peroxisome-proliferator response element (PPRE) in their promoter. An even more dramatic response can take place in yeasts grown on different carbon sources. In glucose, a few small peroxisomes are present, whereas 20–25 large organelles arise when cells are shifted to a growth medium containing a fatty acid as sole carbon source. Important transcription factors in yeast that are responsible for expression of the required genes include Pip2p and Oaf1p, which are both members of the Zn_2Cys_6 family of transcription factors[18,19]. Activation takes place via an oleate response element (ORE) in the promoter of the induced genes. Remarkably, although proteins of the protein-import machinery and many of the peroxisomal enzymes have been conserved from yeast to man, the mammalian (PPAR and RXR) and yeast transcription factors (Pip2p and Oaf1p) have nothing in common. The class of nuclear receptors to which PPAR-α belongs is unknown in yeast, and Zn_2Cys_6 transcription factors are typical of fungi[20].

Peroxisomal metabolism could remove important ligands for PPAR-α. For instance, prostaglandins are metabolized within peroxisomes, and phytanic acid, a branched-chain fatty acid, is degraded in liver peroxisomes. In several diseases caused by peroxisome malfunction, phytanic acid accumulates and activates PPAR-α, thus inducing the transcription of a number of genes containing a PPRE[21]. Such observations illustrate how single mutations affecting peroxisome function can lead to such a perplexing combination of clinical manifestations (see below).

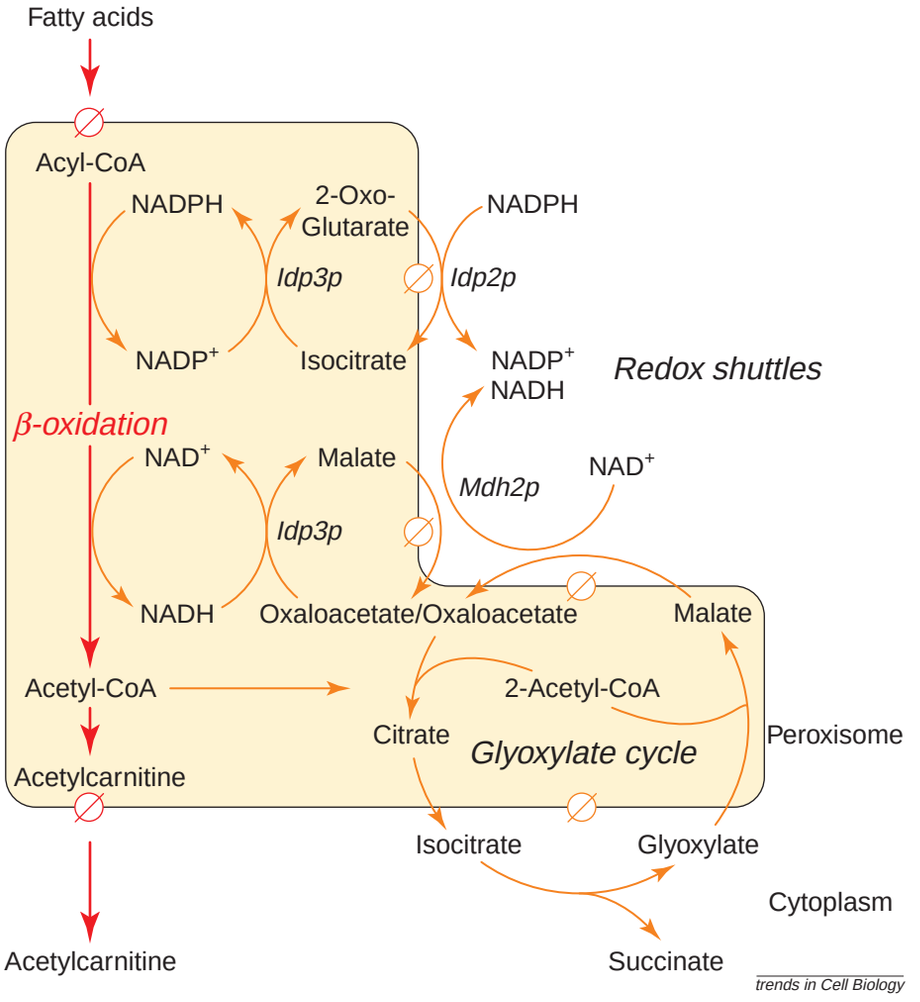

FIGURE 1

Outline of the β-oxidation and accessory metabolic pathways in peroxisomes of *Saccharomyces cerevisiae*. The primary β-oxidation pathway is shown in red, and the accessory pathways that maintain β-oxidation are shown in orange. These comprise reduction equivalents going inwards via NADP and isocitrate/α-oxoglutarate, reduction equivalents going outwards via NAD and malate/oxaloacetate and the export of acetyl-CoA via the glyoxylate cycle or acetylcarnitine pathway. The ∅ symbols indicate the Pat1/Pat2 ABC transporter and other putative transporters that remain to be identified.

Peroxisome maintenance

To maintain peroxisomes in dividing cells, the organelles must be multiplied and segregated to daughter cells. Since membranes are not synthesized *de novo*, peroxisomes can be inherited through one of two mechanisms: new peroxisomes either arise by growth and division of preexisting peroxisomes or the membranes and crucial membrane proteins are recruited from another vesicular compartment such as the endoplasmic reticulum. This matter is hotly debated in the peroxisome field, and in such discussions the term 'biogenesis' has often been used. However, because its use implies *de novo* synthesis, we prefer to use the more neutral term 'maintenance'. Studies in various yeasts (*S. cerevisiae*, *H. polymorpha*, *Candida boidinii*, *Pichia pastoris* and *Yarrowia lipolytica*) have yielded insight into some aspects of maintenance processes, particularly the import of proteins into peroxisomes. Genetic screens make use of a conditional requirement for peroxisomes. Growth of a mutant *S. cerevisiae* cell on a fatty acid (oleate) should be impaired, whereas growth on glucose, glycerol or ethanol remains undisturbed (oleate non-utilizers or *onu* mutants). Among these are mutants with a severe phenotype in which matrix proteins are mislocated in the cytosol. These so-called *pex* mutants provide important information about maintenance functions such as import of matrix proteins into peroxisomes, insertion of membrane proteins into peroxisomal membranes, recruitment of phospholipids to increase the membrane surface, fission and fusion of peroxisomes and segregation of organelles to daughter cells. Fig. 3 gives a schematic overview of our current knowledge about the import of peroxisomal matrix proteins, and Table 1 lists all the *PEX* genes that have been discovered so far.

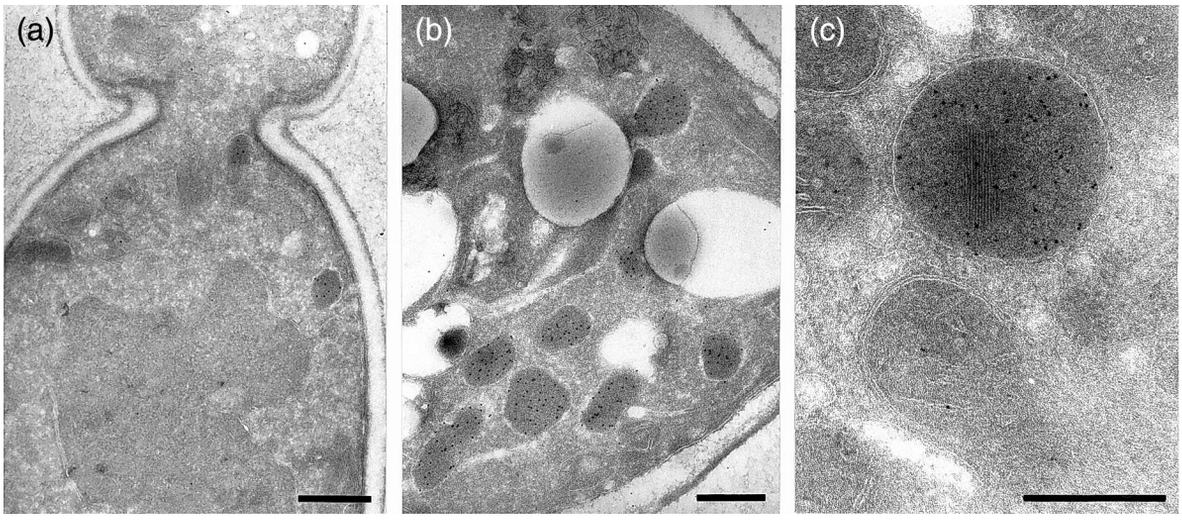

FIGURE 2

Electron micrographs of peroxisomes. (a) and (b) *Saccharomyces cerevisiae* peroxisomes grown on glycerol (a) and the fatty acid oleate (b). (c) Rat liver peroxisome containing a typical crystalloid. Gold particles show the localization of matrix proteins. Bars, 0.5 μm.

After synthesis on free polysomes, peroxisomal matrix proteins are released into the cytosol and recognized by specific receptors for targeting to the peroxisomal membrane. Pex5p recognizes matrix proteins via their C-terminal tripeptide motif (PTS1); Pex7p recognizes an N-terminally located amino acid motif (PTS2). The main components onto which cargo and receptors dock are Pex13p (an integral peroxisomal membrane protein) and Pex14p (a peroxisomal membrane-associated protein), as established by a combination of techniques: yeast two-hybrid interactions, co-immunoprecipitations and, recently, surface plasmon resonance analysis using purified proteins[9,22]. Well-defined (sub)domains exist in many of the Pex proteins (peroxins) for which the three-dimensional structure is known from other protein family members (see Table 1). This allows more extensive analysis of the mechanistic details of the interactions that together result in protein import.

At present, studies are somewhat hampered by the fact that there is no consensus about the behaviour of the various proteins that are implicated in the protein-import process[9,23]. For example, the observation that the PTS1 and PTS2 receptors (Pex5p and Pex7p) have been found in the cytosol, the peroxisomal matrix or both has led to different models for the import of matrix proteins. Most researchers agree about the first part: recognition in the cytosol and docking onto the peroxisomal membrane. Nothing is known about the actual membrane translocation process except that proteins can go in retaining a (partially) folded conformation[24]. This might be why attempts to try to jam the translocon with certain protein constructs to enable crosslinking of the construct to components of the translocation pore have met with failure until recently (see below). Some researchers entertain the view that cargo and receptor go in together (the extended-shuttle model), which can explain why Pex5p and Pex7p are sometimes found in the peroxisomal matrix. However, this model evokes numerous questions. How are Pex5p and Pex7p able to disentangle themselves from the mass of accumulated PTS1 and PTS2 amino acid sequences because these motifs are not processed and removed as in other organelles? Does the translocon also work in reverse, like the translocon in the endoplasmic reticulum (ER) membrane, or can we expect to find an export machinery specifically dedicated to recycle Pex5p and Pex7p back to the cytosol? McNew and Goodman proposed the interesting idea that import might resemble endocytosis[24]. When the lipids of the endocytosed vesicles are subsequently redistributed to the peroxisomal membrane, the net result would be import. An important technical handicap to further progress is the lack of an *in vitro* reconstituted protein-import system similar to those for mitochondria, ER and chloroplasts. Recently, some hope was raised by successful reconstitution steps using plant glyoxysomes and protein constructs that remain jammed in the import site[25].

Even less is known about targeting of membrane proteins to peroxisomal membranes. Some membrane proteins can be inserted in purified peroxisomes in an *in vitro* system[26], and attempts to define a membrane-targeting signal (mPTS) have been reported[27]. How lipids reach the peroxisomal membrane is also an enigma. Here, old ideas of an ER-to-peroxisome connection are being revitalized. These basically originated from observations that, under certain conditions, peroxisomal membrane proteins are located in the ER. This initiated speculation that a vesicular trafficking route might exist for certain peroxisomal membrane proteins and lipids from the ER to peroxisomes. The implications are numerous, but the evidence is scarce. The experiments carried out in *Y. lipolytica*[28] are the only real challenge to the model originally proposed by Lazarow and Fujiki in 1985 that states that peroxisomes multiply by growth and division of pre-existing organelles[29]. The experiments by the Rachubinski group showed that Pex2p and Pex16p are *N*-glycosylated and can be followed in a

pulse–chase experiment from ER to peroxisomes, and it will be important to confirm these results using other organisms.

Other results suggesting an ER–peroxisome connection remain open to alternative interpretations. In certain yeast *pex* mutants, there is no detectable trace of peroxisomes. However, upon transformation of the *pex* mutant with the corresponding wild-type *PEX* gene, peroxisomes reappear even after long-term propagation in the mutant state[30]. What is the source for the reformation of the peroxisomes: the ER? Experiments have been performed with brefeldin A (BFA), which inhibits ARF1-COPI-mediated vesicular transport. In *H. polymorpha*, this resulted in accumulation of some peroxisomal proteins in an ER-like compartment[31]. However, in human fibroblasts, peroxisome formation was not affected by BFA. In these cells, peroxisome formation could be restored in a patient-derived *pex16* cell line by introduction of the wild-type *PEX16* gene – both in the absence and in the presence of BFA[30]. It is difficult to evaluate the many different results because similar experiments have different outcomes when different organisms are used. In addition, *pex* mutants do not always display the same phenotype among the different yeast species and man. For example, there are no residual peroxisomal structures in human *pex16* cells, whereas a similar mutant in *Y. lipolytica* does have residual peroxisome-like structures[32].

The peroxisomal membrane protein Pex11p is implicated in the segregation of peroxisomes to daughter cells[33]. Mammalian Pex11p-α mixed with brain extract specifically binds through its C-terminal di-lysine motif (KXKXY) to coatomer complex (COPI coat) in a GTP- and ARF-dependent manner. Overexpression of Pex11p causes proliferation of peroxisomes, and the authors proposed that Pex11p is instrumental in an ARF-coatomer-dependent vesiculation process of mature peroxisomes. These results are exciting for various reasons. They could not only provide insight into the peroxisome proliferation process but they might also link peroxisomes to the general cellular machinery for vesiculation. Caveats here are that the C-terminal motif in Pex11p that is responsible for recruiting the COPI coat is not conserved among Pex11p from different species and that proliferation of peroxisomes in man was not inhibited by BFA[30].

After a first selection in a genetic screen, most *pex* mutants were characterized biochemically for mislocalization of matrix proteins to the cytosol. In some cases, the *pex* phenotype is, as would be expected, caused by a primary defect in the peroxisomal protein-import machinery[9]. In other cases, the mistargeting is probably an indirect consequence resulting from a primary defect in other aspects of the biogenesis of the organelles. Without peroxisomes, the proteins stay in the cytosol. It is not at all clear in which processes these other Pex proteins act (Table 1); most of them are associated with, or integrated in, (peroxisomal) membranes. Two Pex proteins, Pex1p and Pex6p, belong to the AAA (ATPases associated with diverse cellular activities) family. In

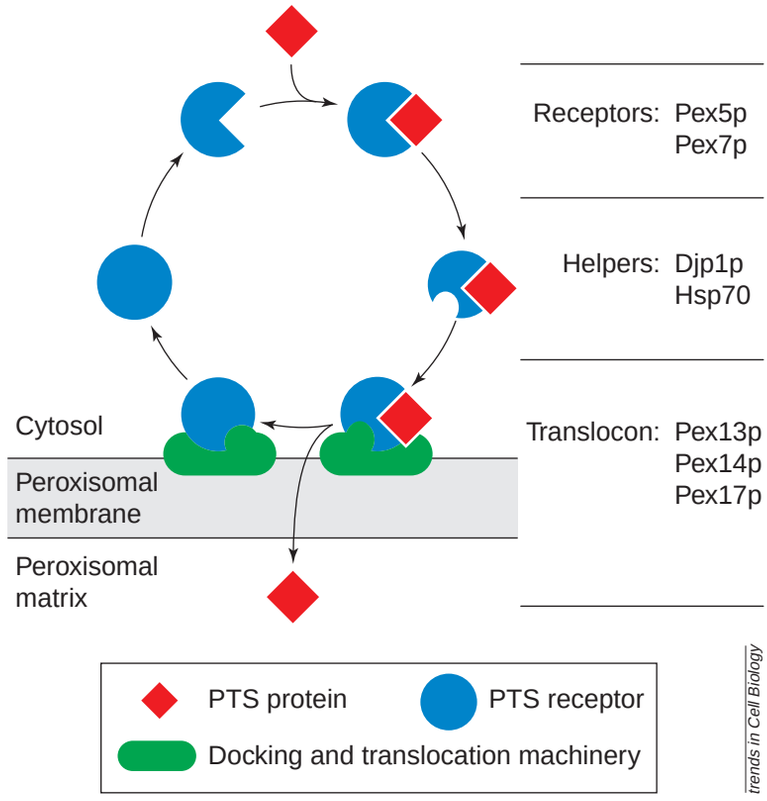

FIGURE 3

A schematic representation of the peroxisomal-targeting sequence (PTS)-dependent protein-import pathway for proteins destined for the peroxisomal matrix, showing the recognition of PTS-containing proteins in the cytosol by soluble receptors and the subsequent targeting of this complex to docking proteins in and on the peroxisomal membrane. Here, we show the most conservative version of the protein-import model, in which receptors recycle from the membrane to the cytosol for a next round of import. It is possible that receptors are internalized together with cargo and that they cycle back to the cytosol from the peroxisomal matrix.

P. pastoris, these proteins interact in an ATP-dependent manner and are associated with vesicles distinct from peroxisomes[34]. They are related in amino acid sequence to NSF (NEM-sensitive factor), an important component of vesicle trafficking in the secretory pathway. Such observations support the idea that biogenesis of peroxisomes is a multistep maturation process in which small pre-peroxisomal vesicles fuse to form the large structures seen by microscopy. Even less is known about Pex2p, Pex10p and Pex12p, except that these peroxisomal membrane proteins contain RING domains, known as protein-interaction modules. However, what part they play, and with what, remains to be discovered.

Finally, there is increasing evidence for the involvement of DnaJ-like and Hsp70-like proteins in the cytosolic phase of protein import into peroxisomes[35,36]. One would expect that such components are part of a general-purpose machinery. Surprisingly, the absence of the cytosolic DnaJ-like protein Djp1p causes defective import of proteins into peroxisomes only; import into the ER, nucleus and mitochondria is unaffected. Despite close scrutiny, solid evidence for protein-folding factors in the peroxisomal matrix is lacking. This might be related to the fact that (partially) folded proteins can be imported. However, certain events that occur in the

TABLE 1 – PEX GENES INVOLVED IN PEROXISOME MAINTENANCE[a]

Gene	Protein characteristics	PBD known	Structural motif
PEX1	Belongs to the family of AAA ATPases	+	
PEX2	Integral membrane protein; contains RING-finger motif	+	C3HC4 zinc-finger
PEX3	Integral membrane protein		
PEX4	Membrane-associated protein; ubiquitin-conjugating enzyme		UBC fold
PEX5	PTS1 receptor; localized to the cytosol and peroxisome; contains TPR repeats	+	TPR motif
PEX6	Belongs to the family of AAA ATPases	+	
PEX7	PTS2 receptor; localized to the cytosol and peroxisome; contains WD40 repeats	+	WD40 motif
PEX8	Peroxisome-associated protein; contains a PTS1 signal		
PEX9	Integral membrane protein		
PEX10	Integral membrane protein; contains RING-finger motif	+	C3HC4 zinc-finger
PEX11	(Integral) membrane protein		
PEX12	Integral membrane protein; contains RING-finger motif	+	C3HC4 zinc-finger
PEX13	Integral membrane protein; contains SH3 domain	+	SH3 domain
PEX14	Membrane-associated protein	+	
PEX15	Phosphorylated integral membrane protein		
PEX16	Membrane-associated protein	+	
PEX17	Membrane-associated protein		
PEX18	PTS2 pathway-specific; interacts with Pex7p		
PEX19	Farnesylated protein associated with peroxisomes	+	
PEX20	PTS2 pathway-specific; interacts with thiolase		
PEX21	PTS2 pathway-specific; interacts with Pex7p		

[a]A listing of proteins involved in peroxisome maintenance. If known, the presence of structural motifs is indicated (SH3, Src-homology 3 domain; UBC, ubiquitin-conjugating family of proteins; AAA, ATPases associated with diverse cellular activities; RING, really interesting new group of proteins containing C3HC4 Zn-finger domains). Relationship with a mutated gene version and a peroxisomal biogenesis disorder (PBD) is represented by +. To avoid confusion, we have not mentioned the nature of the disease. This is caused by the fact that no clear correlation exists between genotype and clinical phenotype. A more elaborate list with data and references is on the WEB:
`www.mips/biochem.mpg.de/proj/yeast/reviews/pex_table.html`

matrix are likely to be dependent on a helping hand by molecular chaperones: these could include association of alcohol oxidase monomers to octamers in *H. polymorpha* or the postulated discharge of PTS cargo from receptors in the matrix followed by return of the empty receptors to the cytosol.

Peroxisomes and disease

As discussed above, the function of peroxisomes can be compromised in two different ways[37]. A mutation can affect a gene coding for a peroxisomal enzyme and thus abrogate an important or less important part of the metabolism of the organelle. For example, adult Refsum's disease is caused by deficient phytanoyl-CoA hydroxylase[38], and primary hyperoxaluria type 1 can be caused by missorting of a single enzyme, alanine:glyoxylate aminotransferase[39]. A single mutation can also affect a gene encoding a product involved in the maintenance of this organelle (peroxisome-biogenesis disorder, PBD). Peroxisomal remnants ('ghosts') can still be found in the cells of such patients, or peroxisomes can vanish completely. It is a miracle that children with these disorders are born at all, which leaves one to wonder how complex developmental processes can still take place and how cells can survive without a typical eukaryotic subcompartment.

Thanks to the fundamental research carried out in yeast on *PEX* genes, a number of human and mouse Pex proteins have been identified in databases searched with the amino acid sequences of the yeast orthologues. In 11 cases, the molecular basis for a peroxisomal disease could be related to a nonfunctional Pex protein. Rhizomelic chondrodysplasia punctata (*pex7*) and the cerebrohepatorenal syndrome of Zellweger (*pex5*) are typical examples of a new and surprising class of diseases that are caused by defects in protein trafficking[40–43]. Although these new insights provide no prospect for therapy, they open up the option of prenatal diagnosis.

A confusing aspect is the far-from-perfect correlation between an affected gene and the classification of peroxisomal diseases made by physicians on the basis of clinical phenotypes. This became apparent when patient fibroblasts were used in cell–cell fusion experiments to identify various complementation groups. Within one complementation group, rescue of peroxisome function cannot take place upon cell–cell fusion, whereas fusion between fibroblasts of different complementation groups restores peroxisome function. Remarkably, different diseases sometimes group within the same complementation group. Diseases such as Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum's disease, for

example, can be caused by mutations in the PTS1 receptor – Pex5p – or in Pex1p, a protein belonging to the AAA family. We need detailed information from structure–function analysis of the Pex proteins and the effect of each particular mutation in order to understand this puzzling discrepancy between medical and molecular-biological classifications.

The great range in the severity of the diseases, combined with the fact that compromised peroxisomal function is not lethal, suggests that many mutations might go unnoticed and merely contribute to a predisposition to disease or to a slight loss of peroxisomal function that forms no basis for complaint. We can safely assume, therefore, that mutated genes coding for certain peroxisomal proteins could be important contributors to multifactorial diseases in which loss of gene function is only exacerbated in combination with additional mutations. An interesting example that might illustrate this is X-linked adrenoleukodystrophy[44]. This disease shows an extremely variable clinical phenotype even within the same family with the same mutation. There is no correlation with the position of the mutation in the adrenoleukodystrophy gene, and this variability is also observed in cases in which the adrenoleukodystrophy protein is not even made. Researchers have speculated about the existence of modifiers, and these could well be cryptic mutations in other genes that, in combination with the adrenoleukodystrophy mutations, enhance the severity of the clinical manifestation of X-linked adrenoleukodystrophy. Time will tell whether peroxisomes are more important to health than we have recognized so far on the basis of the limited number of sporadic, strongly disabling diseases known to us.

In summary

We have highlighted several aspects of peroxisome function that concern their contribution to cellular performance. Flux of reduction equivalents across the peroxisomal membrane is mediated by cytosolic and peroxisomal isoenzymes; carriers are required to take care of exchange of metabolites across the membrane, but only a few have been identified so far. We have reviewed the various ideas circulating in the literature about the origin of new peroxisomes and how they are maintained during cell multiplication. A number of proteins take care of import of matrix proteins into peroxisomes; many interactions between these Pex proteins have been catalogued, but how their concerted action results in protein import remains a puzzle. We have argued that the remarkable conditional requirement for peroxisomes that depends on cell type or external conditions has major implications for human health – that is, (partial) loss of peroxisome function is not lethal but certainly affects the quality of life. Finally, we can expect a new wave of interesting results based on knowledge of complete genome sequences and techniques to visualize global gene expression patterns such as serial analysis of gene expression (SAGE) and DNA microarrays. These techniques might be the perfect tools to obtain deeper insight into multifactorial diseases related to peroxisome malfunction.

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Acknowledgements

We apologize to colleagues whose work could not be cited directly because of limitations of space. We thank Piet Borst, Ronald Wanders, Ewald Hettema and members of our laboratories for fruitful discussions, and Marlene van den Berg for the electron micrographs. Research was sponsored by the Netherlands Organization for Scientific Research (NWO).