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# Peroxisomes: organelles at the crossroads

Ralf Erdmann, Marten Veenhuis  
and Wolf-H. Kunau

*Recent years have seen remarkable progress in our understanding of the function of peroxisomes in higher and lower eukaryotes. Combined genetic and biochemical approaches have led to the identification of many genes required for the biogenesis of this organelle. This review summarizes recent, rather surprising, results and discusses how they can be incorporated into the current view of peroxisome biogenesis.*

Peroxisomes are versatile, single-membrane-bound organelles occurring almost ubiquitously in eukaryotic cells. In humans, defects in their structure and/or function give rise to a group of genetically distinct, mostly fatal, inborn errors, the peroxisomal disorders<sup>1</sup>. The discovery of these disorders about a decade ago triggered a dramatic increase in the interest of cell biologists in this organelle. Originally, peroxisomes were considered to be relic organelles that carried out oxygen metabolism in the primitive ancestors of eukaryotic cells but lack a distinct function in modern organisms. However, it has now been recognized that they are metabolically very active and that cells flexibly adapt peroxisome number, size and protein content to the metabolic needs of different organisms and tissues<sup>2</sup>.

The prevailing view of peroxisome biogenesis has changed substantially during the past 12 years. The original model proposed that peroxisomes are formed by budding from the endoplasmic reticulum (ER)<sup>3,4</sup>. A more recent theory is that new peroxisomes originate by division of pre-existing ones and that organellar growth is accomplished by specific posttranslational import of matrix and membrane proteins<sup>5</sup>. As a consequence, it has been considered that peroxisomes arose initially by endosymbiosis<sup>6</sup> and that peroxisomal protein import occurs in a way mechanistically analogous to that of mitochondria and plastids. Peroxisome biogenesis consists, conceptually, of three aspects: peroxisomal membrane synthesis, import of matrix proteins, and peroxisome proliferation. As a result of new data, models of the mechanism of peroxisome biogenesis are once again in a state of flux. In this review, we describe recent progress in our understanding of the first two aspects of the process in eukaryotic cells in general, particularly yeast cells, and suggest how the latest findings may fit into the generally accepted model of peroxisome biogenesis.

## Model systems for studying peroxisomes

Glyoxysomes in germinating plant seeds and peroxisomes of rat liver were initially the favoured systems for studying functional and structural aspects of these organelles<sup>7</sup>. It was then recognized that, in a variety of yeast species, proliferation of the few peroxisomes seen in cells grown on glucose can be strongly induced by growth on other carbon sources (Fig. 1). This allows regulation of the number of peroxisomes per cell over a wide range by shifting to different media. Based on the assumption that the principles of peroxisome biogenesis are conserved between lower and higher eukaryotes, yeasts therefore seemed promising systems for studies of this process. Another especially advantageous feature of yeast cells is that they can grow normally on rich media in the absence of peroxisomes<sup>8</sup>.

Genetic approaches have led to major breakthroughs in understanding peroxisome biogenesis at the molecular level. The key to the application of genetics for the elucidation of peroxisome biogenesis was the isolation of peroxisome-deficient mutants (*pex*-mutants) from yeast species such as *Saccharomyces cerevisiae*, *Hansenula polymorpha*, *Pichia pastoris* and *Yarrowia lipolytica* and from Chinese hamster ovary cells<sup>9,10</sup> (Fig. 1). These mutants were instrumental to the identification of protein components essential for the biogenesis of peroxisomes in higher and lower eukaryotes.

## Gene products essential for peroxisome biogenesis

The diversity of experimental systems used led to a profusion of names for genes and proteins involved in peroxisome biogenesis (Table 1), including the acronyms *PAS*, *PAF*, *PER*, *PAY*, *PEE* and *PMP*, and an even greater array of numbers. Recently, the nomenclature for the peroxisome-biogenesis factors has been unified, and proteins involved in peroxisome biogenesis are now collectively called 'peroxins', with *PEX* representing the gene acronym<sup>10</sup>.

Most of the *PEX* genes have been identified through corresponding *pex* mutants. Such mutants in yeasts, CHO cells and human fibroblasts show a surprisingly small range of phenotypes in terms of mistargeting of peroxisomal matrix enzymes and of peroxisome morphology<sup>9,11,12</sup>. In most *pex* mutants, the import of matrix proteins containing type I or type II peroxisomal-targeting signals (PTs) is defective, and morphologically the peroxisomes are either aberrant or undetectable. There are only two *PEX* genes known (*PEX5* and *PEX7*) for which a deficiency leads to an import defect in which only one of the two protein-import pathways is impaired<sup>9,11,12</sup>. These genes appear to encode the import receptors responsible for the recognition of PTs (see below). In two cases (*PEX10* and *PEX11*), the phenotype of mutants and overexpression of the corresponding wild-type genes suggest that they are involved in peroxisome proliferation<sup>9</sup>. With one exception, all *pex* mutants characterized so far contain peroxisomal membranes, termed ghosts<sup>13</sup>, indicating that targeting and import of peroxisomal membrane proteins is still functional. No peroxisomal remnants have

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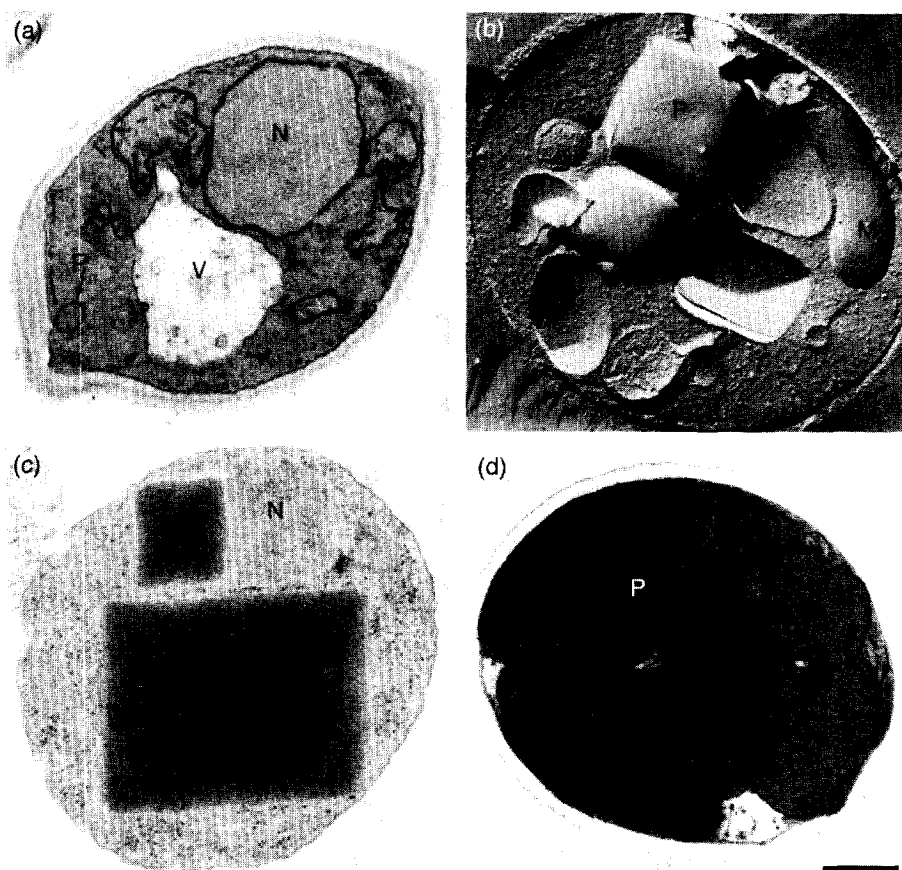
been detected yet in cells lacking the membrane-bound peroxin Pex3p<sup>14</sup>, suggesting that it may be essential for the targeting or import of peroxisomal membrane proteins. Interestingly, *pex3* mutant cells can be functionally complemented by wild-type *PEX3*, leading to the reappearance of peroxisomes<sup>9,14</sup>. If the *pex3* mutant really lacks peroxisomal ghosts, which is difficult to prove, this observation raises intriguing questions about the origin of the peroxisomal membrane (see below) as membranes are generally thought to form only from pre-existing membranes<sup>15</sup>.

None of the peroxins identified so far has been described in other contexts, and they are therefore likely to perform peroxisome-specific roles in the cell. Only in one case sequence analysis suggested the function of the protein – Pex4p appears to be a ubiquitin-conjugating enzyme. A precise function for most of the peroxins has not yet been determined, but some of them are characterized by defined sequence motifs, including the AAA cassette, C<sub>3</sub>HC<sub>4</sub> zinc fingers, WD40 and TPR repeats, which may provide clues to their specific role in peroxisome biogenesis<sup>9,11,12</sup>.

### Peroxisomal-targeting signals

A major contribution to our understanding of peroxisomal protein import was the discovery by Subramani and coworkers of two signal sequences that target proteins to the peroxisomal matrix. The peroxisomal-targeting signal 1 (PTS1) consists of species-specific and protein-context-dependent variations of the tripeptide consensus Ser-Lys-Leu, and this signal comprises the C-terminal three amino acids of most peroxisomal matrix proteins<sup>12,16–18</sup>. The protein-context dependence of the PTS1 may result from peroxisomal matrix proteins being imported in a folded state (see below) – the availability of the targeting signal for recognition could depend on the conformation of the protein. PTS2 signals occur within the first 20–30 amino acids of a subset of matrix proteins. Alignment and site-directed mutagenesis of PTS2-containing proteins led to a PTS2 consensus sequence: Arg/Lys-Leu/Ile-5x-His/Gln-Leu<sup>19</sup>. The PTS2 signal sequence is often cleaved upon import, but this does not seem to be a prerequisite for PTS2-dependent targeting<sup>12,16,17</sup>. Of the peroxins summarized in Table 1, only Pex8p contains an obvious peroxisomal-targeting signal. In fact, Pex8p possesses both a C-terminal PTS1 and an N-terminal PTS2<sup>16</sup>.

There are still open questions concerning the targeting signals of luminal peroxisomal proteins. A four-amino-acid C-terminal PTS belonging to the PTS1 family has been described in human catalase<sup>18</sup>. Also, some peroxisomal matrix proteins lack a consensus



**FIGURE 1**

Ultrastructural appearance of peroxisomes in wild-type and mutant *Hansenula polymorpha* yeast cells grown under peroxisome-proliferation-inducing and non-inducing conditions. (a) Ultrathin section of a non-induced, glucose-grown wild-type cell, showing the overall cell morphology and the typical small peroxisome. (b) Freeze-fractured induced wild-type cell, taken from a methanol-limited chemostat to demonstrate the typical cuboid shape of proliferating peroxisomes. (c) Protoplast of a methanol-induced *pex* mutant, which is characterized by a defect in peroxisome biogenesis. In these mutants, characteristic crystalloids of mislocalized peroxisomal alcohol oxidase form in the cytosol and the nucleus. (d) Induced wild-type cell from a methanol-limited chemostat. Under these growth conditions, the organelles may take up over 80% of the cytoplasmic volume. Electron micrographs are taken from potassium-permanganate-fixed cells, except for (c), which has been fixed with glutaraldehyde and osmium tetroxide. M, mitochondrion; N, nucleus; V, vacuole; P, peroxisome. Bar, 0.5  $\mu\text{m}$ .

PTS1 or PTS2, and others have been reported to be sorted by internal regions that do not resemble PTS1 or PTS2<sup>20</sup>, raising the possibility that there is a third PTS. However, it is not known whether import of these proteins depends on one of the two known signal-recognition factors. This is of particular interest as import of some PTS1-containing proteins still occurs upon deletion of this signal sequence and, despite their lack of an obvious PTS1, import of such proteins still depends on the presence of the PTS1 signal-recognition factor<sup>21,22</sup>. Several explanations could account for this observation. First, the sequence specificity of the PTS1 recognition factor might not be restricted to the C-terminal tripeptide targeting signal, allowing the recognition factor to recognize other sites. In PTS1-containing proteins, these regions might function as accessory binding sites for the PTS1 recognition factor. Second, an additional as-yet-unidentified signal-recognition factor might recognize internal targeting signals and mediate binding to the PTS1 recognition factor.

**TABLE 1 – PEROXISOMAL PROTEINS: CHARACTERISTICS AND FORMER NAMES<sup>a</sup>**

Peroxin	Peroxin characteristics <sup>b</sup>	Former name <sup>c</sup>	Refs
Pex1p	117–127 kDa; belongs to the family of AAA-ATPases; contains two AAA domains; intracellular localization not determined.	ScPas1p	1
Pex2p	35–52 kDa; contains characteristic C <sub>3</sub> HC <sub>4</sub> zinc-finger motif, integral peroxisomal membrane protein.	PpPas1p	2
		RnPAF1	3
		HsPAF	4
		PaCar1p	5
		PpPer6p	6
Pex3p	51–52 kDa; integral peroxisomal membrane protein.	ScPas3p	7
		HpPer9p	8
		PpPas2p	9
Pex4p	21–24 kDa, ubiquitin-conjugating protein, associated with the peroxisomal membrane.	ScPas2p	10
		PpPas4p	11
Pex5p	64–69 kDa; contains at least six TPR motifs; PTS1 recognition factor; localized to the cytosol as well as to the peroxisomal membrane and matrix.	PpPas8p	12
		ScPas10p	13
		HsPxr1p	14
		HsPTS1R	15,16
		HpPer3p	17
		HpPah2p	18
		YIPay32p	19
Pex6p	112–127 kDa; belongs to the family of AAA-ATPases; contains two AAA domains; has been localized to the cytosol.	PpPas5p	20
		ScPas8p	21
		YIPay4p	22
		RnPAF2	23
		HsPXAAA1	24
Pex7p	42 kDa; contains seven WD40 motifs; PTS2 recognition factor; localized to the cytosol as well as to the peroxisomal membrane and matrix.	ScPas7p	25
		ScPeb1p	26
		HsPex7p	27–29
Pex8p	71–81 kDa; contains both a C-terminal PTS1 and an N-terminal PTS2; has been localized to the peroxisomal matrix and inner aspects of the peroxisomal membrane.	HpPer1p	30
		PpPer3p	31
		ScPas6p	32
Pex9p	42 kDa; integral peroxisomal membrane protein.	YIPay2p	33
Pex10p	34–48 kDa; integral peroxisomal membrane protein; contains C <sub>3</sub> HC <sub>4</sub> zinc-finger motif, suggested to be involved in peroxisome proliferation or lumen formation.	HpPer8p	34
		PpPas7p	35
Pex11p	27–32 kDa; peroxisomal membrane protein, involved in peroxisome proliferation; deficiency results in giant peroxisomes.	ScPmp27p	36,37
		CbPmp30p	38
Pex12p	48 kDa; contains a degenerate C <sub>3</sub> HC <sub>4</sub> zinc-finger motif; integral peroxisomal membrane protein.	PpPas10p	39
Pex13p	43 kDa; C-terminal SH3 domain, membrane receptor for the PTS1 recognition factor; putative docking protein for peroxisomal protein import.		40–42
Pex14p	38 kDa, peripheral membrane protein; membrane receptor for both the PTS1 and PTS2 recognition factors. Putative point of convergence of the PTS1- and PTS2-dependent protein-import pathways.		43,44
Pas9p	23 kDa; localized at the cytoplasmic surface of the peroxisome		45
Pas12p	40 kDa; localized in the cytosol as well as the cytosolic surface of peroxisomes. Contains a C-terminal consensus sequence for farnesylation.		46,47
Pas21p	43 kDa; peroxisomal integral membrane protein.		48
Pas22p	48 kDa; cytosolic Dnaj-homologue		48

<sup>a</sup>This table summarizes the currently identified proteins involved in peroxisome biogenesis. According to a unified nomenclature, these proteins are now collectively called peroxins, with PEX representing the gene acronym<sup>10</sup>.

<sup>b</sup>Typical sequence features and, if applicable, the suggested role of the peroxin in peroxisome biogenesis.

<sup>c</sup>Protein names before the nomenclature unification. A PEX number has not yet been assigned to all peroxins; in such cases, these are listed with their Pas nomenclature. Abbreviations: AAA, ATPase associated with diverse cellular activities, PTS, peroxisomal-targeting signal. SH3 domain, Src-homology 3 domain; TPR, tetratricopeptide repeat.

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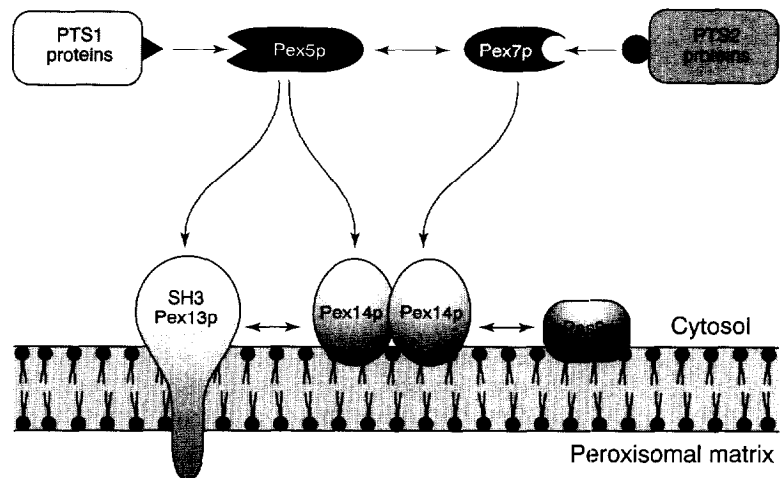
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Finally, the recent finding that proteins can be imported into peroxisomes as dimers or homo-multimers (see below) opens the possibility that they might also get imported as hetero-multimers. Proteins lacking a PTS might then be co-imported with PTS1- or PTS2-containing proteins. An internal dimerization domain of proteins lacking an obvious PTS could then mistakenly be defined as a peroxisomal-targeting signal.

Our understanding of the targeting of peroxisomal membrane proteins is poor largely because only a few of them have been characterized. However, an important feature emerging from current studies is that targeting of peroxisomal integral membrane proteins seems to be PTS1 and PTS2 independent<sup>23-26</sup>, suggesting that special peroxisomal membrane-targeting signals (mPTSs) exist. Indeed, Goodman and coworkers reported that the peroxisomal-targeting information of *Candida boidinii* Pmp47p, which has six transmembrane domains, may be contained within a 20-amino-acid hydrophilic loop between two transmembrane segments<sup>27</sup>. Furthermore, the mPTS of *S. cerevisiae* Pas21p appears to be contained within its C-terminal 82 amino acids (Y. Elgersma, PhD thesis, University of Amsterdam, 1995). By contrast, the first 45 amino acids of *S. cerevisiae* Pex3p (T. Krause, PhD thesis, University of Bochum, 1995), which comprise the putative membrane-spanning domain of this protein, and the first 40 amino acids of *P. pastoris* Pex3p<sup>28</sup>, are sufficient to target a reporter protein to peroxisomes and anchor it into the membrane. Remarkably, the first 16 amino acids of Pex3p from *H. polymorpha* are reported to deliver a reporter protein to the ER<sup>14</sup>. How this observation might fit into a new model of peroxisome biogenesis will be discussed below.

### Components of the peroxisomal protein-import apparatus

Although most of the peroxins summarized in Table 1 are promising candidates for components of the peroxisomal protein-import machinery, evidence for such a function has only been provided for a few of them. The general import deficiencies of peroxisomal matrix proteins observed for many *pex* mutants could be caused indirectly by, for example, defects in peroxisome formation or proliferation. However, Pex5p and Pex7p have been shown to interact directly with PTS1 and PTS2, respectively<sup>12</sup>, and they appear to function as specific signal-recognition factors, or import receptors<sup>11,12</sup>. Recent studies have aimed to define protein components of the protein-import machinery based on their interaction with these two import receptors<sup>23-26</sup>. These have led so far to the identification of three membrane-bound peroxins, proposed to be components of the docking and/or translocation complex of the import machinery for peroxisomal matrix proteins. A summary of interactions between the import receptors and the newly identified putative components of the peroxisomal protein-import machinery from *S. cerevisiae*, based on two-hybrid binding studies, is shown in Figure 2. The PTS1 receptor, Pex5p, binds to the cytosolic Src-homology 3 (SH3) domain<sup>23-25</sup> of the



**FIGURE 2**

Interaction of putative components of the peroxisomal protein-import machinery based on the two-hybrid methodology. Proteins harbouring one of the two peroxisomal-targeting signals, PTS1 or PTS2, are recognized in the cytosol by specific signal-sequence-recognition factors, so-called import receptors (Pex5p and Pex7p). Pex13p and Pex14p present binding sites for the PTS1 receptor at the peroxisomal membrane. Pex14p interacts with both the PTS1 and the PTS2 receptor and may be a point of convergence of the two pathways. Pex14p also interacts with Pex13p, as well as with Pas9p, and these proteins could form a permanent or transient heteromeric docking complex at the peroxisomal membrane. A peroxisomal protein-import cascade in which the cargo-receptor complex is transferred from one component of the translocation machinery to the next is discussed in the text. How protein translocation proceeds through the peroxisomal membrane has not yet been resolved. Abbreviation: SH3, Src-homology 3 domain.

integral membrane protein Pex13p<sup>23-25</sup>. Pex14p, a peripheral peroxisomal membrane protein, interacts with both the PTS1 and the PTS2 receptor<sup>26</sup>. Furthermore, Pas9p, a newly discovered membrane-bound peroxin, interacts with Pex14p and with the PTS1 receptor<sup>26</sup>. Pex14p also binds to Pex13p, and again the binding is mediated through the SH3 domain of Pex13p. Pex14p also self-associates, suggesting that it homo-oligomerizes *in vivo*<sup>26</sup>. The two-hybrid system is prone to generating false positives, particularly for membrane-bound proteins<sup>29</sup>. Thus, these results need to be confirmed by independent methods. Such additional evidence has been provided for the interaction of Pex5p and Pex13p as well as for the interaction of Pex5p, Pex7p, Pex14p and Pas9p by co-immunoprecipitation and *in vitro* binding studies<sup>23-26</sup>.

The two import receptors Pex5p and Pex7p and the three membrane-bound peroxins Pex13p, Pex14p and Pas9p are currently the only identified components of the import machinery for peroxisomal proteins. However, not every *in vivo* interaction would be identified by the two-hybrid approach, and additional peroxins may well be components of the peroxisomal protein-import machinery. The discriminating import defect observed for the *pex8-1* mutant strain from *P. pastoris* suggests that Pex8p is directly involved in protein import<sup>30</sup>, and circumstantial evidence supports Pex2p and Pex12p being components of the peroxisomal protein-import machinery as defects in these two proteins result in the accumulation of the PTS1 receptor at the peroxisomal membrane in human fibroblasts<sup>31</sup>.

Two explanations might account for the multiple interactions observed for some of the putative components of the import machinery. The interacting components might be part of heteromeric complexes involved in protein import. Alternatively, as not only a permanent but also a transient *in vivo* association can account for a two-hybrid interaction, the multiple interactions might reflect the existence of an import cascade involving these peroxins. It can be hypothesized that the cargo proteins remain bound to their signal-recognition factors, which during at least part of the translocation process might be transferred from one import component to the next.

#### **Protein import into peroxisomes: breaking the rules?**

Accumulated evidence, reviewed by Lazarow and Fujiki<sup>5</sup>, supports the hypothesis that peroxisomal matrix proteins are synthesized on free ribosomes and imported posttranslationally into pre-existing organelles<sup>5</sup>. The energy and cytosol requirements of the import process have been addressed by various experiments, primarily with cells from higher eukaryotes<sup>32-34</sup>. Although at present only a rough framework for peroxisomal protein import has been established, it appears not to be just another version of the well-characterized translocation processes of mitochondria or the ER – new principles seem to apply.

First, it is essential to establish the subcellular localization of the components involved. This situation is puzzling as, depending on species and experimenter, the localization of the import receptors ranges between cytosolic, membrane associated and intraperoxisomal<sup>11,12,17</sup>. These conflicting data suggest that the PTS receptors may have a dynamic rather than static distribution. This would imply that the receptors have a more complex role in peroxisomal protein import than simply recognition of PTS-containing proteins. One possibility is that PTS receptors might not only collect their cargo proteins in the cytosol and then direct them to the peroxisome but, in addition, might carry them across the membrane into the peroxisomal matrix, then shuttle back to the cytosol. The steps of this model that take place in the cytosol and at the cytosolic face of the peroxisomal membrane are supported by experimental evidence. Pex13p and Pex14p provide the required binding sites for the PTS receptors at the outer face of the peroxisomal membrane<sup>23-26</sup>. Furthermore, in fibroblast cell lines, the PTS1 receptor accumulates on or near the surface of peroxisomes when protein translocation is blocked either by loss of putative translocation factors or by low-temperature incubation and/or ATP depletion<sup>31</sup>. This situation is reversible: Pex5p is released to the cytosol and accumulates on the peroxisome again when the translocation-inhibiting conditions are released and then re-enforced<sup>31</sup>. However, it is not yet clear whether receptor–ligand dissociation takes place at the translocation site or inside the peroxisomal matrix. Both PTS receptors have been reported inside the peroxisome in different yeasts<sup>11,12</sup> as has the PTS1 receptor in a fibroblast cell line derived from a patient with a peroxisome-biogenesis disorder (PBD)<sup>31</sup>. However, at present, it cannot be determined whether

this intraperoxisomal accumulation of PTS receptors is due to impaired dissociation at the translocation site, which then might result in an 'artificial' import, or to an inhibition of the export of the receptor. Thus, although accumulating data are consistent with the model of shuttling PTS receptors, the distinction between a short shuttle (between cytosol and peroxisomal surface) and extended shuttle (between cytosol and peroxisomal matrix) is far from resolved. An alternative view suggesting that the physiological site of action of the PTS receptors is solely inside the peroxisomes<sup>35,36</sup> can still not be ruled out, but, from the accumulating data, this seems unlikely.

One question that was left open in recent models for peroxisomal protein import, concerning whether the PTS1- and PTS2-dependent protein import into the peroxisomal matrix is carried out by distinct or common import sites, now seems to be answered. The observations that Pex14p has the ability to interact with both the PTS1 and the PTS2 receptor<sup>26</sup> and that lack of either Pex13p or Pex14p leads to a general import defect for peroxisomal matrix proteins<sup>23-26</sup> provide strong evidence for the existence of common protein-import sites for the matrix proteins. However, targeting and insertion of integral membrane proteins seem not to require the matrix protein-import machinery, suggesting that an independent import pathway for membrane proteins exists. This is consistent with the apparent existence of distinct targeting signals for peroxisomal membrane proteins and with the presence of peroxisomal membrane ghosts in *pex13* and *pex14* mutants, suggesting that they can still target and import peroxisomal membrane proteins<sup>23-26</sup>.

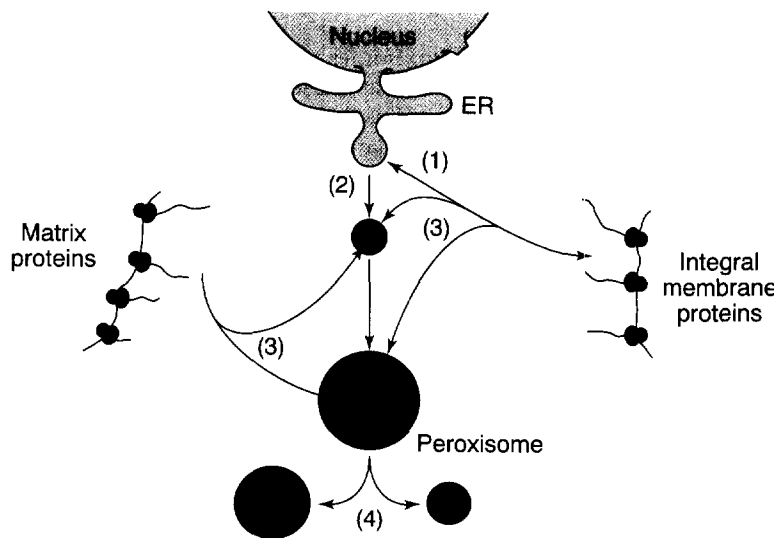
Evidence from several laboratories suggests that pre-folded, even oligomeric, proteins, including albumin crosslinked to PTS1 peptides, dihydrofolate reductase fusion proteins complexed with aminopterin, dimeric thiolase and malate dehydrogenase, and trimeric chloramphenicol acetyltransferase, can be transported into the peroxisomal lumen<sup>16,37</sup>. Even disulfide-bonded IgG molecules and 9-nm gold particles can be imported into peroxisomes when decorated with PTS1-resembling peptides<sup>38</sup>, and detailed studies on the interaction of the PTS2 recognition factor and thiolase led to the conclusion that both proteins are folded<sup>39</sup>. No peroxisomal chaperones have yet been identified despite extensive searches, consistent with folding of peroxisomal proteins occurring prior to their import. The ability to import folded proteins and oligomers suggests that the peroxisomal membrane contains pores capable of accommodating these large structures. However, up to now, there is no evidence for the existence of such pores. On the contrary, studies by the Veenhuis and Tabak groups suggest that the permeability properties of peroxisomes are not consistent with the existence of large pores, unless these do not allow leakage of small metabolites<sup>40,41</sup>. McNew and Goodman suggested that peroxisomal protein import might involve a new form of endocytosis at the peroxisomal membrane<sup>42</sup>, but this interesting idea is not supported by experimental evidence, and it raises new questions concerning the fate of the invaginated membranes and release of the vesicular contents.

Another intriguing question relates to the differential import competence of peroxisomes. There is evidence that heterogeneity exists between peroxisomes within one cell with respect to their capacity to incorporate newly synthesized proteins<sup>43-45</sup>. Based on *in vivo* observations in *H. polymorpha* and *C. boidinii*, van der Klei and Veenhuis suggested that there are special protein-import sites on the peroxisomal membrane that are donated to newly formed organelles during fission, resulting in import-competent new organelles and import-incompetent mature ones<sup>45</sup>. This would be consistent with the difficulty of establishing reliable and efficient *in vitro* systems for the import of matrix proteins into mature yeast peroxisomes. However, it is in conflict with *in vivo* experiments with mammalian cells that do not show selective import in part of the peroxisomal population<sup>46</sup>.

**Peroxisome proliferation: new evidence for an old point of view?**

As discussed above, the potential involvement of the ER in peroxisome biogenesis has been a controversial topic throughout the history of peroxisome research. Experimental evidence clearly shows that peroxisomal membrane and matrix proteins are synthesized on free ribosomes in the cytosol and imported posttranslationally into peroxisomes<sup>5</sup>, all of which supports a model in which these organelles originate by division of pre-existing peroxisomes. However, an involvement of the ER in at least one step of peroxisome biogenesis, namely the formation of the peroxisomal membrane, cannot be ruled out.

This new but still hypothetical view is incorporated into the model of peroxisome biogenesis depicted in Figure 3. The new model still incorporates most features of the previous conception of peroxisome biogenesis but seeks to explain recent data that are hard to reconcile with the earlier view. At least one peroxisomal membrane protein has been reported to be synthesized on membrane-bound ribosomes<sup>47</sup>. The idea that some peroxisomal membrane proteins become associated with the ER prior to their transport to peroxisomes provides an explanation for the observation that, upon overexpression of the *S. cerevisiae* peroxisomal membrane proteins Pex3p and Pas21p, ER-like endomembranes significantly proliferate and peroxisomal membrane proteins are mistargeted to the nuclear envelope, and also that Pas21p truncated at the C-terminus is mistargeted to the plasma membrane (Y. Elgersma, PhD thesis, University of Amsterdam, 1995; T. Krause, PhD thesis, University of Bochum, 1995). Moreover, incubation of exponentially growing *H. polymorpha* on methanol in the presence of brefeldin A (BFA) prevented peroxisome formation and resulted in decoration of the ER with peroxisomal matrix proteins<sup>48</sup>. In addition, the first 16 amino acids of *H. polymorpha* Pex3p have been reported to target a reporter protein to the ER<sup>14</sup>. These new data need to be interpreted with caution as they might represent experimental artifacts, but equally they might reflect the existence of an as-yet-undefined route for transport of peroxisomal membrane proteins from the ER to peroxisomes. The peroxisomal membrane proteins that are targeted



**FIGURE 3**

Hypothetical model for peroxisome biogenesis. (1) A subset of peroxisomal membrane proteins, probably peroxins involved in the early stages of peroxisome biogenesis, is inserted into the endoplasmic reticulum (ER) membrane. (2) Vesicles harbouring these peroxisomal membrane proteins bud from the ER and fuse with peroxisomes. (3) Peroxisomal matrix proteins and other peroxisomal membrane proteins are synthesized on free ribosomes in the cytosol and imported posttranslationally into peroxisomes by different pathways. (4) Peroxisomes grow and undergo fission to form new peroxisomes. Although conceptually very attractive and suggested by the data discussed in the text, this model now needs to be substantiated by experimental data.

initially to the ER might be involved in the earliest stages of peroxisome biogenesis<sup>12</sup>. If these 'early' peroxins are essential for the biogenesis of the peroxisomal membrane, cells lacking these proteins would be expected not to contain peroxisomal membrane ghosts. Such a phenotype has been described for yeast cells lacking Pex3p<sup>14</sup> and for fibroblasts of PBD complementation group 9 (Ref. 31). But how would these, initially ER-resident, 'early' peroxins reach their peroxisomal destination? It is conceivable that vesicle-mediated transport from the ER to peroxisomes occurs, similar to that originally proposed by Goldman and Blobel in 1978 (Ref. 49). Pre-peroxisomal transport vesicles might be generated by budding from the ER; these could then fuse heterotypically with pre-existing peroxisomes or undergo homotypic fusion to form new peroxisomes. In this regard, it is interesting to note that deficiency in Pex1p and Pex6p results in accumulation of small peroxisomal vesicles that contain minute amounts of peroxisomal matrix proteins<sup>13,50,51</sup>. Pex1p and Pex6p belong to the AAA (ATPases associated with diverse cellular activities) family of proteins, members of which recently have been shown to be involved in homo- and heterotypic fusion in the Golgi apparatus and ER<sup>52-54</sup>. The AAA peroxins might be involved in a vesicle-fusion step essential for peroxisome assembly, which could be the hetero- or homotypic fusion of pre-peroxisomal vesicles derived from the ER. Subsequently, newly formed peroxisomes or pre-peroxisomal vesicles might import matrix proteins and other membrane proteins posttranslationally to form functional peroxisomes. The proposed involvement of ER-derived vesicles in peroxisome biogenesis might also explain the heterogeneous

ability of peroxisomes to import newly synthesized proteins<sup>43-45</sup>. Pulse-chase experiments with mammalian cells indicated that a peroxisomal compartment of intermediate density is the primary target for newly synthesized acyl CoA oxidase<sup>43</sup>. Furthermore, upon microinjection of alcohol oxidase into mammalian cells, the recruitment of a protein constituent of the ER into peroxisome-like vesicles that are import-competent for alcohol oxidase has been reported<sup>55</sup>. In view of these data, the decoration of the ER with peroxisomal matrix proteins in the presence of brefeldin A might be interpreted as indicative of the assembly of peroxisomal protein-import sites at the ER<sup>48</sup>. However, to avoid peroxisomal matrix proteins being imported erroneously into the ER lumen, these import sites would need to remain inactive until incorporated into the transport vesicles.

### Concluding remarks

The past few years have seen the combination of genetics, biochemistry and morphology speed up the advancement of our understanding of peroxisome biogenesis. Nevertheless, although 18 genes encoding peroxins have been identified, we still know very little about how most of these proteins participate in peroxisome biogenesis.

Following the discovery of peroxisomes, the mechanisms involved in their biogenesis were thought to be a simple variation of that of other organelles. However, recent evidence suggests that new rules apply. At present, the prevailing view of peroxisome biogenesis is in a transition phase. The aim of modified models such as the one depicted in Figure 3 is to highlight urgent questions rather than to give conclusive answers. The involvement of the ER in peroxisome formation is an attractive speculation that might explain some observations that are hard to reconcile with the current view on peroxisome biogenesis. Furthermore, it also raises new questions, such as whether the protein components of the vesicle-mediated transport in the secretory pathway are also involved in peroxisome biogenesis. As most of these components are essential, they would not have been identified by the screening for *pex* mutants.

A pathway for the transport of membrane proteins from the ER to peroxisomes would have far-reaching consequences for our understanding of peroxisome biogenesis as it would open the possibility for *de novo* synthesis of peroxisomes, as has been suggested to occur in *Arthobotrys oligospora* and *H. polymorpha*<sup>56,57</sup>. Acquiring experimental evidence for the existence and nature of vesicle-mediated transport from the ER to peroxisomes will certainly be a key challenge for future research.

The ultimate challenge in peroxisome biogenesis research is still the complete elucidation of the mechanisms of peroxisomal matrix and membrane protein import. In particular, the problem of how folded or even oligomerized proteins traverse the peroxisomal membrane still remains. This, in fact, is the clearest indication that the mechanisms underlying peroxisome biogenesis are significantly different from what we currently know about other subcellular organelles. It is now clear that the basic

molecular features of peroxisome biogenesis are conserved in lower and higher eukaryotes. The fungal *PEX* genes provide an attractive starting point for identifying their counterparts in humans and therefore open a promising avenue for investigating the molecular genetics of the human peroxisome-biogenesis disorders<sup>58</sup>.

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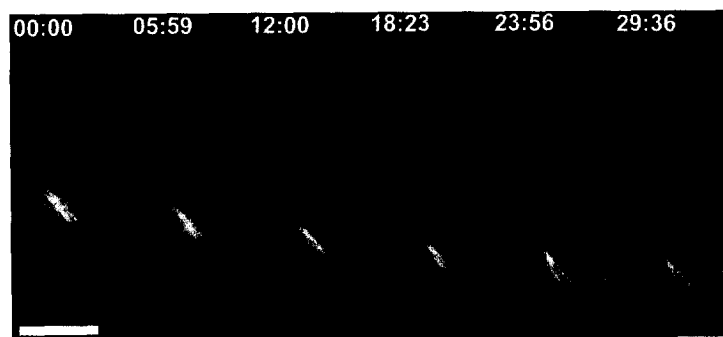


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## Pictures in cell biology

### Microtubule dynamics in migrating cells

The classic conception that the microtubule (MT) cytoskeleton in interphase cells is organized by a perinuclear MT-organizing centre, the centrosome, from which all MTs emanate out towards the cell periphery, is currently under scrutiny. The MT cytoskeleton of non-motile cells is often thought of as a rigid framework that provides structural support for the cell and tracks for the movement of organelles to and from the cell periphery. A recent study examined how the MT cytoskeleton responds to cell migration and found that MT dynamics and organization in motile cells differ considerably from those reported for stationary cells<sup>1</sup>. MT dynamics were visualized by microinjecting fluorescently labelled tubulins into migrating newt lung epithelial cells. The dynamics of MTs in the lamella differed depending on their orientation with respect to the leading edge of the cell. By marking the MT lattice by photoactivation (Fig. 1), the authors also discovered that MTs in the lamella move continuously rearward towards the cell centre, not unlike the well-studied retrograde flow of the cell surface and of the actin cytoskeleton in motile cells. In fact, rearward movement of MTs occurs at the same rate as cell-surface movement and is sensitive to inhibitors of actin and myosin but not to nocodazole, which inhibits MT dynamics. Retrograde flow of MTs in these cells was associated with MT buckling and breaking in the lamella. MT breakage generates a population of MTs with stabilized minus-ends that are not bound to the centrosome, as well as some MTs that shorten at their minus-ends and treadmill through the lamella. In fact, the authors found that only ~20% of the MTs in these cells are centrosome bound. These observations suggest that the actin–myosin system has profound effects on the dynamics and arrangement of MTs during cell movement. The next step will be to pursue the basis of these phenomena at the molecular level.



**FIGURE 1**

Retrograde movement of the lattice of microtubules (MTs) in the lamella of a migrating newt lung epithelial cell. Digitally overlaid and pseudocoloured micrographs of a cell that was injected with a mixture of tubulins labelled with X-rhodamine (red) and caged fluorescein (yellow-green). The cell was exposed to a bar of UV light 2.5  $\mu\text{m}$  wide to activate the fluorescein label and thus mark a segment of the MTs just prior to acquisition of the first image. The leading edge of the cell is at the top of the image, and the nucleus is to the lower right of the field of view; the cell was migrating towards the top of the page. Time elapsed (in minutes:seconds) is in the upper left of each panel. The fluorescein-

labelled subunits in MTs that are primarily perpendicular to the leading edge of the cell move rearward through the lamella, while the X-rhodamine-labelled portion of the MTs distal to fluorescein marks grows over time. This shows that the lattice of MTs in the lamella is continuously moving rearward towards the cell centre. Bar, 10  $\mu\text{m}$ . URL for movies: <http://www.unc.edu/depts/biology/salmon.html>.

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