

Review Article

Peroxisome Dynamics: Molecular Players, Mechanisms, and (Dys)functions

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Peroxisomes are remarkably versatile cell organelles whose size, shape, number, and protein content can vary greatly depending on the organism, the developmental stage of the organism's life cycle, and the environment in which the organism lives. The main functions usually associated with peroxisomes include the metabolism of lipids and reactive oxygen species. However, in recent years, it has become clear that these organelles may also act as intracellular signaling platforms that mediate developmental decisions by modulating extraperoxisomal concentrations of several second messengers. To fulfill their functions, peroxisomes physically and functionally interact with other cell organelles, including mitochondria and the endoplasmic reticulum. Defects in peroxisome dynamics can lead to organelle dysfunction and have been associated with various human disorders. The purpose of this paper is to thoroughly summarize and discuss the current concepts underlying peroxisome formation, multiplication, and degradation. In addition, this paper will briefly highlight what is known about the interplay between peroxisomes and other cell organelles and explore the physiological and pathological implications of this interorganellar crosstalk.

1. Introduction

Peroxisomes are single membrane-bounded cell organelles that can be found in all nucleated cells. These organelles, originally described as “microbodies,” were first visualized in cytological studies of mouse proximal kidney tubules [1] and rat hepatocytes [2]. In 1966, de Duve and Baudhuin carried out the first detailed biochemical investigations on freshly isolated “microbodies” from rat liver and kidney and introduced the term “peroxisome” to refer to the organelle's central role in the production and degradation of hydrogen peroxide (H_2O_2) [3]. Over the years, our knowledge and understanding of how this organelle functions within the cell has gradually increased. For example, it has turned out that a major function of peroxisomes in all organisms is to regulate cellular lipid metabolism. In addition, it has become clear that the enzymatic content of these organelles (and hence their functions) can vary substantially across species. This is best illustrated by the fact that certain organisms contain specialized peroxisomes that are named differently. For example, germinating seeds of plants contain “glyoxysomes”, a subclass of microbodies that contain enzymes of the glyoxylate cycle

[4]; members of the protist order Kinetoplastida contain “glycosomes”, a category of specialized peroxisomes that compartmentalize the enzymes of the glycolytic pathway [5]; filamentous fungi contain “woronin bodies”, a class of peroxisome-derived vesicles that seal the septal pore in response to wounding [6]. However, also “peroxisomes” themselves may house species-specific metabolic pathways. For example, in the fungus *Penicillium chrysogenum*, these organelles harbor enzymes crucial for the synthesis of β -lactam antibiotics [7]; in methylotrophic yeasts, they harbor the key enzymes of methanol metabolism [8]; in plants, they play a key part in jasmonic acid and auxin synthesis [9, 10]; in mammals, they play a pivotal role in the biosynthesis of bile acids and plasmalogens [11]. Finally, there is growing evidence that (mammalian) peroxisomes are not solely metabolic organelles but may also act as signaling platforms that sense and integrate signals arising from viral pathogens and age-related processes [12–14]. For a detailed description of peroxisome function in the major model organisms, I refer to other excellent reviews [8, 15–21].

The pivotal role of peroxisomes in eukaryotic organisms is perhaps best underscored by the existence of a group of

genetic disorders associated with peroxisomal deficiencies. These disorders are generally grouped into two broad categories: the peroxisome biogenesis disorders (PBDs) and the single peroxisomal enzyme deficiencies (PEDs) [22]. The PBDs result from a failure in peroxisome assembly and include three major clinical phenotypes that represent a continuum of clinical features that are most severe in Zellweger syndrome (OMIM 214100), milder in neonatal adrenoleukodystrophy (OMIM 202370), and least severe in infantile Refsum disease (OMIM 266510) [23]. Interestingly, despite the fact that these PBDs currently encompass 14 distinct genes, no clear genotype-phenotype correlation has been established [23]. The main reasons for this are most likely the nature of the mutations in the involved genes (see below) and peroxisome mosaicism [24]. The PEDs are a group of disorders in which the peroxisomal structure is intact and functioning, except for a single metabolic pathway [22]. Intensive efforts by multiple research groups have led to the identification of many PED-causing mutations in at least 10 distinct peroxisomal genes [25]. The prototype of this group of disorders is X-linked adrenoleukodystrophy (OMIM 300100), which is the most common (incidence: 1:17000 newborns) of the peroxisomal disorders [26]. Note that all diseases caused by partial or complete peroxisome dysfunction are characterized by a variety of neurological abnormalities [27].

Currently, there is ample evidence that peroxisomes are dynamic organelles that rapidly assemble, multiply, and degrade in response to nutritional and environmental stimuli [28]. In addition, it is becoming increasingly clear that these organelles cooperate with the endoplasmic reticulum (ER) and mitochondria to carry out their functions [29, 30]. This review provides a detailed overview of the molecular players and mechanisms involved in peroxisome formation, multiplication, and degradation (Figure 1). Furthermore, it will summarize recent advances in our understanding of how defects in the dynamic behavior of these organelles can have a negative impact on an organism's functions.

2. Molecular Players

Peroxisomes are highly plastic organelles that can rapidly modulate their size, number, and enzyme content in response to changing environmental conditions. The most impressive examples of this kind are undoubtedly the observations that (i) peroxisomes in *Hansenula polymorpha* can occupy up to 80% of the cytoplasmic volume when this methylotrophic yeast species is cultivated in a medium containing methanol as a sole carbon source [8], and (ii) on recultivation in glucose- or ethanol-containing medium, the vast majority of these methanol-induced organelles are rapidly and selectively degraded because their activity is no longer needed [31]. A similar phenomenon, albeit much less pronounced, can also be observed in rodents upon the administration and subsequent withdrawal of a variety of xenobiotics, collectively referred to as peroxisome proliferators [32]. Importantly, the accurate control of peroxisome density depends on a balance between their synthesis, multiplication, and degradation. Each of these processes

requires the coordinated action of various proteins, which are thought to be organized in large complexes. The identity and properties of these proteins are discussed in the following sections.

2.1. Peroxisome Biogenesis. The formation of new peroxisomes can be viewed as the integration of three processes: the assembly of the peroxisomal membrane, the import of matrix proteins, and proliferation of the organelles [33]. Proteins that are uniquely involved in one of these processes are called “peroxins” (abbreviated “Pex”, and including a number corresponding to the order of their discovery; gene acronym: *PEX*) (Table 1) [34]. Over the years, it has become clear that a core set of these proteins, which can be grouped into distinct classes (see below), is conserved across many species [20]. In addition, it has become evident that various steps in peroxisome biogenesis require the function of proteins that are also involved in other processes [35, 36]. This section is intended to provide up-to-date information on key factors involved in peroxisomal membrane and matrix protein import (the protein complexes that control peroxisome division are described in Section 2.2). Topics that are discussed include the *cis*-acting targeting signals that direct newly formed proteins from their place of synthesis to the peroxisomal compartment (see Section 2.1.1) and the molecular machines that recognize and translocate proteins across or into the peroxisomal membrane (see Sections 2.1.2–2.1.8). For mechanistic models of how peroxisomes may arise *de novo* or from preexisting organelles, I refer to Section 3.2.

2.1.1. Peroxisome Targeting Signals. The assembly of functional peroxisomes requires the import of approximately 100 different nuclear-encoded proteins (for an up-to-date list of datasets, please visit <http://www.peroxisomedb.org/>). These proteins can reside in the peroxisomal membrane or be confined to the matrix of the organelle. Currently, it is generally accepted that all peroxisomal matrix proteins are synthesized on free polyribosomes in the cytosol and posttranslationally imported into preexisting organelles [37]. For membrane proteins, the situation is more complicated; depending on the protein and the organism under study, the biosynthesis of this class of molecules has been reported to occur on free or ER-bound ribosomes [38–43]. Importantly, these findings have significant implications for the mechanisms underlying peroxisome biogenesis (for more details, see Section 3.2).

The vast majority of peroxisomal matrix proteins in virtually all eukaryotic organisms contain a C-terminal peroxisomal targeting signal, called PTS1 [44]. Originally, this targeting signal was defined as an uncleaved tripeptide with the consensus sequence $-(S/A/C)-(K/R/H)-(L/A)^*$ (in single-letter amino acid code; the asterisk represents a stop codon) [45]. In the meantime, this consensus sequence has been broadened, and it has become clear that also residues upstream of the tripeptide may modulate its functionality [46–48]. The molecular mechanisms underlying this phenomenon can be traced to Pex5p, the PTS1 import receptor (see Section 2.1.2). As Pex5p molecules from different species may exhibit a different affinity towards various PTS1

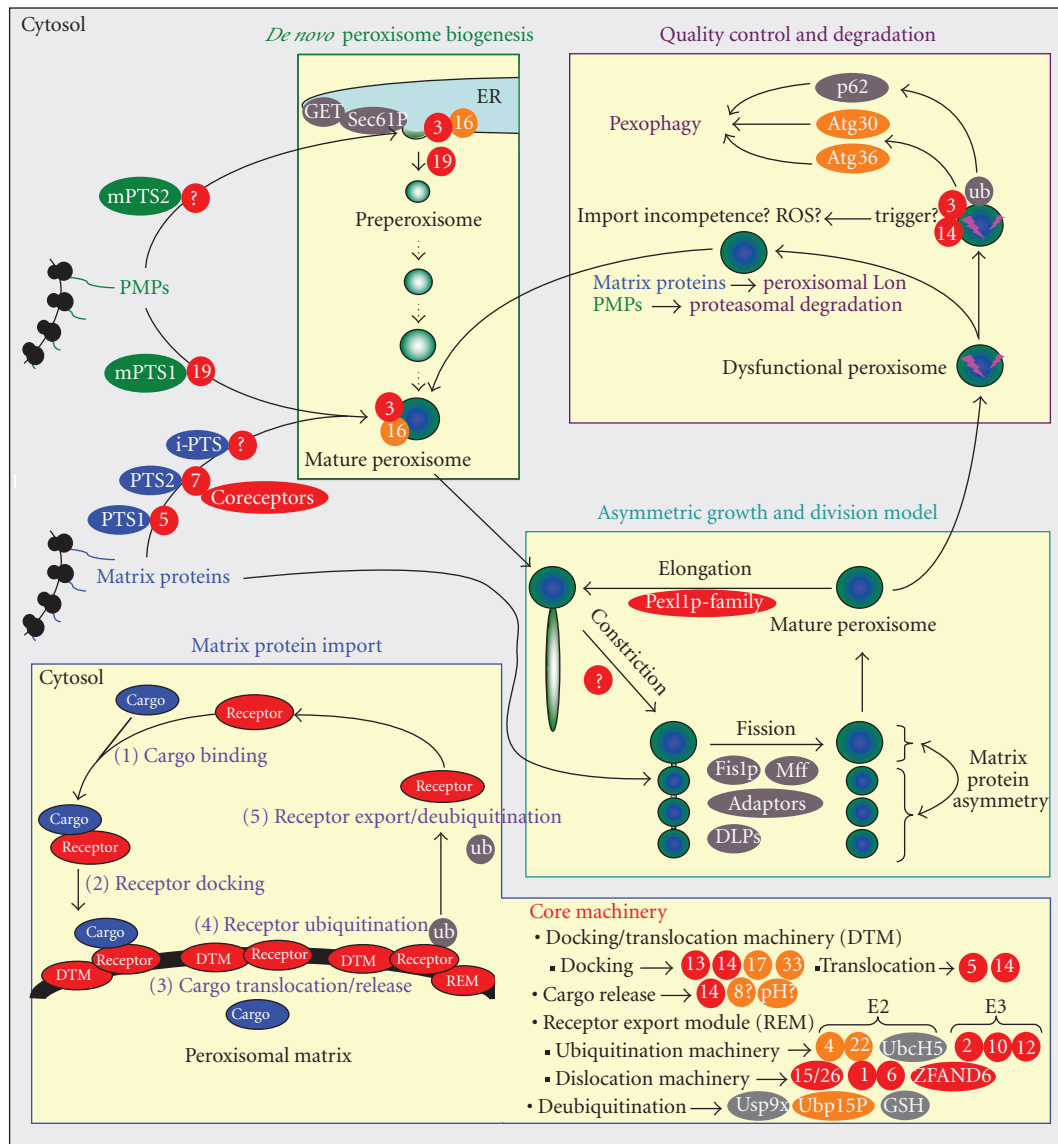


FIGURE 1: Model of peroxisome dynamics. New peroxisomes can be formed *de novo* from the ER or by asymmetric growth and division of preexisting organelles. In the *de novo* pathway, a select set of PMPs is inserted into the ER via the Sec61p translocon (or the GET complex) and sequestered into specialized Pex3p-containing ER exit sites from which smoothed vesicles are pinched off in a Pex19p-dependent manner. These peroxisomal precompartments subsequently develop into mature peroxisomes that are capable of importing matrix proteins. The latter process can be divided into several different stages: cargo-recognition by a PTS receptor; docking of the PTS receptor-cargo complexes at the peroxisomal membrane (the Pex7p-cargo complexes require auxiliary factors for import); cargo translocation across the peroxisomal membrane and cargo release into the peroxisomal matrix; receptor recycling. The latter event requires the involvement of the receptor export module, which ubiquitinates the receptor and extracts it from the peroxisomal membrane. Peroxisomes can also grow in number and size by a complex asymmetric multistep maturation pathway, a process that involves peroxisome elongation, membrane constriction, and organelle fission. Members of the Pex11p-family of peroxins are involved in the elongation process, the components of the constriction machinery are not yet known, and the fission machinery comprises soluble dynamin-like proteins that are recruited to the peroxisomal membrane by Fis1p and/or Mff (with or without the help of adaptor proteins). Importantly, as “old” matrix proteins are retained within the mother organelle, this leads to matrix protein asymmetry. During their life cycle, peroxisomes are also subject to rigorous quality control: oxidatively damaged matrix proteins are degraded by a peroxisomal Lon protease, superfluous PMPs are extracted from the peroxisomal membrane and degraded by the proteasome, and dysfunctional organelles are removed by pexophagy. The triggers and mechanisms underlying the quality control of peroxisomes have only recently begun to be elucidated. Note that (i) peroxins are indicated in white numbers in red (evolutionarily conserved peroxins) or orange (species-specific peroxins) circles; (ii) grey circles represent factors that are also involved in nonperoxisomal processes; (iii) more details can be found in the text.

TABLE 1: List of peroxins identified to date. Peroxins described in mammals are shown in bold. Note that mammalian genomes encode three Pex11 proteins: Pex11 α , Pex11 β , and Pex11 γ . All abbreviations are defined in the “Abbreviations” section.

Peroxin	Features	Functions
Pex1p	AAA-ATPase	Pex5p recycling, fusion of preperoxisomal vesicles
Pex2p	PMP, RING domain	Subunit of the E3 complex for polyubiquitination of Pex5p
Pex3p	PMP	Docking factor for Pex19p, initiation of macropexophagy
Pex4p	Peripheral PMP	Subunit of the E2 complex of for monoubiquitination of Pex5p
Pex5p	TPR protein	PTS1 import receptor, PTS2 coreceptor
Pex6p	AAA-ATPase	Pex5p recycling, fusion of pre-peroxisomal vesicles
Pex7p	WD40 protein	PTS2 receptor
Pex8p	Peripheral PMP	Pex5p-cargo release factor? Organizer of the protein import machinery?
Pex9p		Erroneously identified open reading frame
Pex10p	PMP, RING domain	Subunit of the E3 complex for mono- and polyubiquitination of Pex5p
Pex11p	(peripheral) PMP	Peroxisome elongation factor (peroxisome proliferation)
Pex12p	PMP, RING domain	Subunit of the E3 complex for monoubiquitination of Pex5p
Pex13p	PMP, SH3 domain	Subunit of the PTS-receptor docking complex
Pex14p	PMP	Subunit of the PTS-receptor docking complex, initiation of macropexophagy
Pex15p	PMP	Docking factor for Pex6p
Pex16p	PMP	Docking factor for Pex3p
Pex17p	PMP	Subunit of the PTS-receptor docking complex
Pex18p		PTS2 co-receptor
Pex19p	Farnesylation motif	Chaperone and import receptor for PMPs
Pex20p		PTS2 co-receptor
Pex21p		PTS2 co-receptor
Pex22p	PMP	Subunit of the E2 complex of for monoubiquitination of Pex5p
Pex23p	PMP	Peroxisome proliferation
Pex24p	PMP	Peroxisome proliferation
Pex25p	PMP	Peroxisome proliferation
Pex26p	PMP	Docking factor for Pex6p
Pex27p	PMP	Peroxisome proliferation
Pex28p	PMP	Peroxisome proliferation
Pex29p	PMP	Peroxisome proliferation
Pex30p	PMP, dysferlin domain	Peroxisome proliferation
Pex31p	PMP, dysferlin domain	Peroxisome proliferation
Pex32p	PMP	Peroxisome proliferation
Pex33p	PMP	Subunit of the PTS-receptor docking complex
Pex34p	PMP	Peroxisome proliferation

sequences, some PTS1 variants may act in a species-specific fashion [46]. Note that PTS1 prediction programs that deal with different substrate specificities between fungal, metazoan, and plant PTS1-targeted proteins are available online at <http://mendel.imp.ac.at/pts1/> [49] and <http://ppp.gobics.de/> [50].

The import of another subset of peroxisomal matrix proteins is mediated by a type 2 peroxisomal targeting signal (PTS2) [51]. This signal, which in most (but not all) organisms is cleaved off after it enters the peroxisomal lumen, can be found in the N-terminal portion of a limited number of proteins and consists of a nonapeptide with the “consensus” sequence -R-(L/V/I/Q)-X₂-(L/V/I/H)-(L/S/G/A)-X-(H/Q)-(L/A)- (where X can be any amino acid) [52]. Recently, a detailed mutational study revealed that, in order to be functional, this motif has to form a well defined α -helical structure with a conserved charge distribution [53]. The

molecular mechanisms underlying this phenomenon can be traced to Pex7p, the PTS2 import receptor (see Section 2.1.2). Importantly, some organisms (e.g., *Caenorhabditis elegans*, *Drosophila melanogaster*, and diatoms) lack a functional PTS2 protein import system [21, 54, 55]. However, the orthologues of proteins known to contain a PTS2 signal in other species have acquired a PTS1 in these organisms [21, 54, 55].

Interestingly, a few proteins destined for the peroxisome lumen are targeted to the organelle by unusual non-PTS1/PTS2 sequences [56–60]. However, these internal targeting signals, sometimes termed “i-PTS”, remain largely uncharacterized and are most likely heterogeneous in sequence. Nevertheless, there is experimental evidence that at least some of these sequences may function as oligomerization domains mediating association with other proteins bearing a PTS1 [61–63]. In this context, it is crucial to point

out that the peroxisomal matrix protein import machinery can accommodate the import of folded proteins [64], homooligomeric protein complexes [65, 66], and even nondeformable 9 nm gold particles conjugated to peptides bearing a PTS1 [64]. Nonetheless, the potential physiological relevance of this so-called piggyback import mechanism has not yet been fully resolved and, recently, considerable debate has erupted over whether the peroxisomal import machinery prefers to import monomeric or oligomeric substrates [67, 68].

The biogenesis of peroxisomal membrane proteins (PMPs) is a complex process that requires their targeting to and insertion into the peroxisomal membrane. The *cis*-acting protein sequences guiding this multistep process are called “mPTSs” [69]. These mPTSs, which consist of a targeting element and a membrane-anchoring sequence, vary greatly in length, are unremarkable by primary structure analysis, and are not proteolytically removed upon import [70]. An mPTS can be located at virtually any position within the protein [71]. In naturally occurring proteins, this sorting determinant is positioned near the N-terminus in type 1 PMPs (1 transmembrane segment, $N_{\text{matrix}}\text{-}C_{\text{cytoplasm}}$) and near the C-terminus in type 2 PMPs (1 transmembrane segment, $N_{\text{cytoplasm}}\text{-}C_{\text{matrix}}$) [70]. Interestingly, many polytopic PMPs contain multiple nonoverlapping mPTSs [70]. As virtually all these mPTSs contain binding sites for Pex19p [72], and this protein functions as a chaperone and import receptor for newly synthesized PMPs (see Section 2.1.2), it has been hypothesized that polytopic PMPs may have nonoverlapping mPTSs to ensure their solubility before membrane insertion [73]. Alternatively, the presence of multiple mPTSs may play a role in determining the orientation of the membrane-spanning segments. Finally, there is growing evidence that various PMPs traffic to peroxisomes via the ER [41, 43], and that the mPTSs of some of these PMPs display an overlap with ER targeting signals [74, 75]. These and other (see Section 2.1.2) findings suggest the existence of multiple classes of mPTSs [70, 76, 77]. However, much work remains to be done before definite conclusions can be drawn.

2.1.2. PTS Receptors. In order to be functional, PTSs need to be recognized by specific “import receptors” that guide newly synthesized proteins to and across the peroxisomal membrane. Until now, three such receptors have been identified in a variety of species: Pex5p, Pex7p, and Pex19p. Each of these proteins is discussed in detail in the following paragraphs.

Pex5p functions as a cycling import receptor for newly synthesized PTS1 proteins and some—but not all—non-PTS1/PTS2 proteins [78]. It recognizes and binds these proteins in the cytosol and transports them to the peroxisomal matrix. This process relies upon a complex network of transient protein-protein interactions, including cargo recognition, docking of the cargo-loaded receptor on the peroxisomal membrane, and cargo translocation and release [79]. To fulfill these functions, all Pex5p orthologues contain multiple conformationally flexible segments: in their C-terminal halves, these proteins contain two clusters of three tetratricopeptide repeat (TPR) motifs, which are connected

by a flexible hinge region (previously identified as “TPR4”) and form a single binding site for PTS1 [80, 81]; in their N-terminal halves, they possess multiple diaromatic pentapeptide motifs (often referred to as “WXXXF/Y”), which constitute high affinity binding sites for Pex13p and Pex14p (see Sections 2.1.4 and 2.1.6) [82–84]. The number of these motifs varies among species, ranging from two in *Saccharomyces cerevisiae* to nine in *Arabidopsis thaliana* [85]. Interestingly, both the C- and N-terminal parts of Pex5p undergo substantial conformational changes upon binding to their ligands [86, 87]. So far, little is known about the (internal) region in Pex5p that is involved in binding to non-PTS1/PTS2 cargo proteins [58, 78, 88]. In addition, there is still no consensus whether Pex5p functions as a monomer or tetramer (for more details, see 2.1.6.) [86, 89, 90]. Finally, it should be noted that mammalian cells, as well as some plant cells, contain two isoforms of Pex5p, termed Pex5pS (the short isoform) and Pex5pL (the long isoform), which are generated through alternative splicing [91, 92].

Pex7p is a soluble protein that serves as the targeting signal recognition factor for newly synthesized PTS2 proteins [51]. The protein displays a cytosolic and intraperoxisomal distribution pattern [93, 94] and can be repeatedly translocated in and out of the organelle [95]. All Pex7p orthologues are characterized by the presence of six WD40 repeats, which—together with a distinct N-terminal region—are predicted to form a seven-bladed β -propeller-like structure [96]. Mutations affecting the conformation of this structure almost always abolish activity [94, 97, 98]. Interestingly, although the structure of Pex7p is unknown, recent three-dimensional structural modeling studies of Pex7p revealed a groove with an evolutionarily conserved charge distribution complementary to PTS2 signals [53]. Importantly, the formation of import-competent PTS2 receptor complexes requires the help of accessory molecules (see Section 2.1.3). Note that Pex7p orthologues are absent in organisms lacking a functional PTS2 pathway (see Section 2.1.1).

Pex19p is a predominantly cytosolic, partially peroxisomal multifunctional protein that plays a central role in the early steps of peroxisomal membrane synthesis [99]. The observations that this peroxin can (i) bind a wide variety of newly synthesized PMPs in the cytosol [100], (ii) keep these PMPs in a membrane insertion-competent conformation [42], (iii) transport them to the peroxisomal membrane [73], and (iv) shuttle back to the cytosol [101] have led to the proposal that Pex19p functions as a chaperone and soluble import receptor for “class I” PMPs [73, 99]. Members of this class of PMPs contain common Pex19p-binding motifs that are an integral part of their targeting signals [72, 102, 103]. Interestingly, many PMPs contain multiple Pex19p-binding sites [70, 72], and not all these sites can be directly linked to peroxisomal targeting [69, 71]. However, as many of these binding sites overlap with a predicted transmembrane segment [70], they may serve to recruit Pex19p to exposed hydrophobic patches thereby preventing PMP aggregation and degradation [100, 104, 105]. Note that the binding of Pex19p to some PMPs may also serve to regulate the interaction of membrane-associated protein complexes [69, 106] or to drive the production of preperoxisomal vesicles

(for more details, see Section 3.2) [107–109]. Finally, it should be mentioned that all Pex19p orthologues, with the exception of trypanosomatids [110], contain a prenylation motif at their C-terminus [99]. Nevertheless, despite the observations that Pex19p is (at least partially) farnesylated *in vivo* [105, 111] and that this posttranslational modification may affect the conformation and binding properties of the protein [71, 112], it remains debatable under which conditions and to what extent farnesylation affects the function of Pex19p in peroxisome biogenesis [100, 112–115].

2.1.3. PTS2 Coreceptors. As already mentioned above (see Section 2.1.2), the PTS2 receptor Pex7p requires auxiliary factors for its function in peroxisomal protein import. These auxiliary factors, called “PTS2 coreceptors” [116], are species-specific and include (i) a splice form of Pex5p that resembles Pex5pL, the long isoform of mammalian Pex5p (see Section 2.1.2) (e.g., in animals, plants, and trypanosomatids) [85, 91, 92, 117], (ii) Pex18p or Pex21p (e.g., in *S. cerevisiae* and *Candida glabrata*; both proteins are weakly homologous and display partial functional redundancy) [118, 119], and (iii) Pex20p (e.g., in *Yarrowia lipolytica*, *Pichia pastoris*, *Hansenula polymorpha*, and *Neurospora crassa*) [120–123]. All these proteins are largely cytosolic and display a low overall similarity between their primary sequences [116]. Nevertheless, despite this, they share three structurally and functionally conserved modules: a Pex7p-binding domain, one or more diaromatic pentapeptide motifs that constitute putative binding sites for Pex13p and/or Pex14p (see Section 2.1.4), and an N-terminal region containing a cysteine residue essential for their recycling from the peroxisome to the cytosol (see Section 2.1.7) [124–126]. Unfortunately, it is still unclear at which step(s) these “coreceptors” exert their function in PTS2 import. These may include (i) the oligomerization of PTS2 proteins in the cytosol [120], (ii) the targeting of PTS2 proteins to peroxisomes [120, 122, 127], (iii) the targeting of Pex7p-PTS2 complexes to the peroxisomal membrane [118], and (iv) the translocation of cargo-laden Pex7p across the peroxisomal membrane [128]. The latter possibility will be discussed in detail below (see Section 3.3).

2.1.4. PTS-Receptor Docking Complexes. The peroxisomal membrane contains specific protein complexes that act as docking sites for cargo-laden PTS receptors. Currently, two such complexes have been identified: one recognizing incoming Pex5p- and Pex7p-cargo complexes, and one binding Pex19p-cargo complexes. The composition and function of each of these complexes will be discussed below. Conceptual models of how these complexes may be regulated are presented elsewhere (see Sections 3.2 and 3.3).

The delivery of matrix proteins to peroxisomes requires docking of the Pex5p/Pex7p-cargo complexes to the peroxisomal membrane. This process is mediated by the peroxisomal membrane proteins Pex13p and Pex14p, which form the core components of the docking machinery [129, 130]. Although it is known that Pex5p and Pex7p have multiple binding sites for both Pex13p and Pex14p [86, 131, 132], there is strong evidence that the latter peroxin functions

as the initial docking factor for the cargo-bound PTS-receptors. This is perhaps best exemplified by the observations that (i) cargo-loaded Pex5p displays a higher affinity for Pex14p than for Pex13p [84, 133], and (ii) the amount of peroxisome-associated Pex5p is proportional to the amount of Pex14p [124]. Interestingly, in organisms in which the PTS2 import pathway functions independently of Pex5p (see Section 2.1.3), Pex14p also acts as the point of convergence for PTS1- and PTS2-dependent protein import [131]. Importantly, in these organisms, the peroxisomal docking complex contains a third component, which—based on certain structural and functional characteristics—is called Pex17p [134, 135] or Pex33p [136, 137]. Both peroxins are essential for efficient PTS1 and PTS2 matrix protein import. Note that Pex33p, originally termed Pex14/17p [136], has the properties of both Pex14p and Pex17p: its N-terminus interacts with Pex5p via a highly conserved Pex5p-binding region that is also present in the N-termini of Pex14p proteins; its C-terminus shows weak similarity to Pex17p proteins [136, 137]. The functions of Pex33p and Pex14p are not redundant [137].

It is already known for a long time that, in many species, cells deficient in Pex3p, Pex16p, or Pex19p lack identifiable peroxisomal membrane structures [99]. This observation has led to the hypothesis that these peroxins are essential for peroxisome membrane biogenesis. In the meantime, it has become clear that Pex19p functions as a chaperone and soluble import receptor for class I PMPs (see Section 2.1.2). In addition, there is a wealth of evidence that the peroxisomal membrane protein Pex3p serves as the docking factor for cytosolic Pex19p-PMP complexes: Pex3p specifically interacts with the docking domain of Pex19p [138, 139]; the Pex3p-Pex19p interaction is essential for the peroxisomal localization of Pex19p, and a mislocalization of Pex3p to other subcellular organelles is sufficient to recruit Pex19p to heterologous membranes [138]; Pex3p displays a much higher affinity for cargo-laden Pex19p than for Pex19p alone [42]. The latter observation suggests the existence of a cargo-induced peroxisomal targeting mechanism for Pex19p. Finally, it has been shown that—at least in mammals—the peroxisomal membrane protein Pex16p serves as a docking factor for Pex3p [77].

2.1.5. Translocons. Currently, little is known about how peroxisomal proteins are translocated across or inserted into the organellar membrane. However, recent studies in the yeasts *P. pastoris* and *S. cerevisiae* have provided some initial answers to longstanding questions about the composition of the minimal translocon for peroxisomal matrix protein import [140, 141] and the identity of the translocon for PMPs that travel to peroxisomes via the ER [43]. In addition, functional studies of human Pex3p in combination with mutational analysis have led to a model for PMP import into peroxisomes [142]. Each of these findings is discussed below.

By using different approaches, it has been shown that the PTS1-dependent import of *P. pastoris* Pex8p into peroxisomes requires only Pex5p and Pex14p [140]. In addition, it has been reported that the affinity-purified *S. cerevisiae* orthologues of Pex5p and Pex14p can form a gated

ion-conducting channel upon reconstitution in proteoliposomes, and that this channel can expand in diameter when cytosolic Pex5p-cargo complexes are encountered [141]. Combined, these findings indicate that membrane-associated Pex5p and Pex14p form the key components of a transient matrix protein translocation pore at the peroxisomal membrane. However, it remains enigmatic how this pore can accommodate the transport of folded and even oligomeric cargo proteins without compromising the permeability barrier of the peroxisomal membrane (see Section 3.3).

Currently, there is substantial (but sometimes conflicting) evidence that some PMPs traffic to peroxisomes via the ER, while others are sorted to these organelles directly from the cytosol (see Section 2.1.1). As both transport pathways are mechanistically different, it is reasonable to assume that—depending on the pathway used—multiple translocons of differing compositions are involved. In this context, it is essential to mention that two studies in *S. cerevisiae* have reported that PMPs which traffic to peroxisomes via the ER insert into the latter organelle via the Sec61p translocon or the GET complex [43, 143]: the Sec61p translocon is a multisubunit protein-conducting channel which mediates the transport of the majority of polypeptides destined for the secretory pathway across the ER membrane and the lateral integration of transmembrane segments into the membrane lipid bilayer [144]; the GET complex mediates the posttranslational insertion of tail-anchored proteins into the ER membrane [145]. Importantly, another study reported that a loss of Sec61p activity in this organism had no effect on peroxisome biogenesis [146]. Finally, it is not understood how Pex3p and Pex16p may act in concert to recognize incoming Pex19p-PMP complexes from the cytosol and mediate PMP insertion into the peroxisomal membrane. There is also no consensus whether or not these processes require a source of energy [77, 99]. Recently, it has been suggested that a highly conserved hydrophobic groove on the surface of Pex16p-anchored Pex3p may actively participate in the PMP insertion process [142]. The precise mechanisms of action remain to be elucidated.

2.1.6. PTS Receptor-Cargo Release Factors. While the interactions between the PTS receptors Pex5p, Pex7p, and Pex19p and their cargo proteins are relatively well characterized [53, 80, 147], very little (e.g., for Pex5p-PTS1) or virtually nothing (e.g., for Pex7p-PTS2 and Pex19p-mPTS) is known about how these complexes are dissociated upon delivery of the cargo at their destination. Many years ago, it was proposed that Pex8p, an intraperoxisomal protein containing both PTS1 and PTS2 signals, may act as a PTS1 receptor-cargo release factor [148]. This conclusion was based on the observation that this peroxin can dissociate Pex5p-PTS1 peptide complexes, even in the absence of its PTS1. However, as Pex8p is only present in fungi [119], such a mechanism cannot be operative in higher eukaryotes. In this context, it is interesting to note that the same authors proposed that the dissociation of the Pex5p-PTS1 complex may also be driven by a change in pH [148]. This hypothesis was derived from the following findings in *H. polymorpha*: Pex5p exists in

different oligomeric conformations, and these conformations vary with pH (at pH 6.0, the protein is monomeric; at pH 7.2, it is tetrameric); PTS1 peptides predominantly bind to tetrameric Pex5p; the peroxisomal matrix is slightly acidic [148, 149]. Again, as (i) depending on the organism and overall cell function, the pH of the peroxisomal lumen may vary considerably [150], and (ii) in mammals, soluble Pex5p functions as a monomeric protein [86, 151], other factors than pH may trigger the cargo release step. In the meantime, it has become clear that Pex5p undergoes conformational changes upon cargo-protein binding [152]. This indirectly implies that changes in the conformation of the PTS1 binding site (e.g., upon the interaction of Pex5p with other molecules near the peroxisomal membrane) may result in cargo release. Interestingly, such a role has recently been attributed to Pex14p, the initial docking site for Pex5p at the peroxisomal membrane (see Section 2.1.4). Indeed, Azevedo and coworkers have shown that the binding of this peroxin to the 6th (and 7th) WXXXF/Y motif of Pex5p (see Section 2.1.2) triggers the release of the PTS1 cargo protein [67]. Finally, it has been shown that the release of a cargo into the organellar matrix occurs prior to Pex5p ubiquitination (see Section 2.1.7) [153].

2.1.7. PTS (Co)Receptor Ubiquitination. About a decade ago, it was discovered that *S. cerevisiae* Pex5p is transiently ubiquitinated at the peroxisomal membrane [154–156]. This finding, together with the former observations that also *S. cerevisiae* Pex18p and Pex21p can be ubiquitinated *in vivo* [97], boosted the research in the field of peroxisomal matrix protein import [157]. In the meantime, it is clear that ubiquitination of Pex5p (or any other PTS2 coreceptor) is an evolutionarily conserved process that functions to export the receptor back into the cytosol (see Section 2.1.8) or to dispose the protein when it gets stuck in the import pathway [123, 158, 159]. The former process requires monoubiquitination of Pex5p, Pex18p, Pex20p, or Pex21p at a conserved cysteine residue in their N-terminus [128, 158, 160–162]. The latter process depends on polyubiquitination of (single or multiple) lysine residues [128, 161], a process which forces rapid turnover by proteasomal degradation [163]. In this section, I will focus on the molecular players involved in Pex5p ubiquitination.

The conjugation of ubiquitin-like molecules to a protein requires the concerted action of an ATP-requiring ubiquitin-activating enzyme (E1), a ubiquitin conjugating enzyme (E2), and a ubiquitin-ligase (E3) [163]. In general, an organism's genome encodes only one or two ubiquitin-specific E1 enzymes, a few dozen E2 enzymes, and hundreds of E3 ligases [164]. Many E3 enzymes contain a zinc-binding structural motif of 40 to 60 amino acids, called “RING finger”, and act in multiprotein complexes [165]. Over the years, it has become clear that in all organisms studied so far the peroxisomal membrane contains three putative integral membrane E3s (*in casu* Pex2p, Pex10p, and Pex12p) which contain a RING domain within their cytoplasmically exposed C-terminus [119]. Currently, it is known that the RING domains of these peroxins can form a heteromeric complex and exhibit E3-ligase activity *in vitro* [166–168]. In addition,

it has been demonstrated that Pex10p, which serves as a central organizer of this complex [169], also interacts with Pex4p (sometimes termed Ubc10), a putative E2 enzyme [170]. In the meantime, it has been shown that Pex4p is anchored on the peroxisomal membrane by the integral membrane peroxin Pex22p [171], and that the Pex4p-Pex22p complex and the Pex10p-Pex12p heterodimer function, respectively, as the E2 and E3 enzyme for Pex5p monoubiquitination [166–168, 172]. Interestingly, the Pex4p-Pex22p complex is only present in yeasts and plants, and—in mammalian cells—its function has been taken over by members of the UbcH5 family [173]. Finally, it has been shown that Ubc4p (the yeast homologue of UbcH5) and the Pex2p-Pex10p heterodimer act, respectively, as E2 and E3 enzymes for Pex5p polyubiquitination [166–168]. In summary these findings indicate that the mono- and polyubiquitination events of Pex5p require different sets of E2 and E3 enzymes. Note that mutations in any of these proteins result in a defect in peroxisomal matrix protein import [170, 174, 175].

2.1.8. PTS (Co)Receptor Recycling. To keep the protein import cycle running, the (ubiquitinated) PTS (co)receptors have to be exported back to the cytosol. Unfortunately, virtually nothing is known about how this process works for Pex7p, the PTS2 import receptor (see Section 2.1.2), and Pex19p, the import receptor for class I PMPs (see Section 2.1.2). However, over the years, it has become clear that Pex1p and Pex6p, two peroxins belonging to the AAA type family of ATPases, function as the core components of a complex which dislocates ubiquitinated Pex5p (or any other ubiquitinated PTS coreceptor) from the peroxisomal membrane back to the cytosol [123, 126, 176, 177]. Pex1p and Pex6p can form homo- and heterooligomeric complexes and cycle between the cytosol and the peroxisomal membrane [178, 179]. Both processes are ATP dependent [177, 180]. The Pex1p-Pex6p complex is recruited to the peroxisomal membrane and associated with the PTS1 (co-)receptor ubiquitination machinery through Pex15p (e.g., in baker's yeast) or Pex26p (e.g., in mammals) [181, 182]. The latter two proteins are tail-anchored PMPs which most likely fulfill orthologous functions [119]. Importantly, the dislocation of ubiquitinated PTS (co)receptors from the peroxisomal membrane requires the help of a bridging protein. The first description of such a protein was only recently provided by Fujiki's group, who reported that—in mammals—ZFAND6 functions as a cytosolic adaptor protein between Pex6p and the cysteine-ubiquitinated form of Pex5p [183]. To complete the transport cycle, ubiquitinated Pex5p needs to be deubiquitinated. In this context, it is interesting to note that in *S. cerevisiae* the peroxisomal export machinery is also associated with Ubp15p, a ubiquitin hydrolase that is capable of cleaving off ubiquitin moieties from Pex5p [184]. In mammals, the ubiquitin-Pex5p thioester conjugate is a substrate for USP9x, a cytosolic ubiquitin-specific protease [185]. Finally, it has been shown that, once in the cytosol, the ubiquitin-Pex5p thioester conjugate can also be efficiently disrupted by physiological concentrations of glutathione [186].

2.2. Peroxisome Division. Over the last decade, much progress has been made in our knowledge of how peroxisomes grow and divide from preexisting organelles. Currently, there is a general consensus that this process involves three morphologically distinct steps: peroxisome elongation, constriction of the peroxisomal membrane, and fission of the organelle. Each of these steps will be described in detail below.

2.2.1. Elongation Factors. In all organisms studied to date, the expression levels of members of the Pex11p family of proteins are directly correlated with the number of peroxisomes, even in the absence of extracellular stimuli [187–189]. Since then, it has been shown that members of this protein family act as peroxisome elongation/tubulation factors [190–192]. Interestingly, the genome of virtually every eukaryotic organism encodes multiple Pex11p-related proteins, which are designated differently in distinct organisms [119]. For example, in mammals and in *S. cerevisiae*, these proteins are, respectively, termed “Pex11p α , Pex11p β , and Pex11p γ ” and “Pex11p, Pex25p, and Pex27p” [188]. All Pex11p-related proteins behave as peripheral or integral membrane proteins. However, their membrane topology is not entirely clear and may even vary among species [192]. In addition, it is not yet precisely known whether these different proteins, which can form homo- and heterooligomers [191, 192], fulfill (partially) overlapping functions or exert specific functions under specialized conditions (e.g., under circumstances that require peroxisome proliferation) [193]. Another long-standing question in the field is how Pex11p molecules can deform and elongate the peroxisomal membrane. Some recent studies, carried out by van der Klei's group, found that (i) members of the Pex11p family contain an evolutionarily conserved sequence at their N-terminus which can adopt the structure of an amphipathic helix, (ii) the binding of this helix to negatively-charged membrane vesicles can drive curvature and tubulation, (iii) mutations abolishing the membrane remodeling activity of this helix also hamper the function of Pex11p in peroxisome fission *in vivo*, and (iv) the membrane-binding activity of Pex11p is required for the suborganellar localization of PMPs [194, 195]. In summary, these findings suggest that the insertion of amphipathic helices of Pex11p-related proteins into the peroxisomal membrane can change its local composition and drive curvature and tubulation. Finally, it should be mentioned that many members of the Pex11p family act in concert with other peroxins involved in the regulation of peroxisome size and number. The current list of these peroxins includes Pex28p, Pex29p, Pex30p, Pex31p, Pex32p, and Pex34p [196–199]. The exact function of these proteins in peroxisome division remains to be elucidated.

2.2.2. Constriction Factors. Until today, it is unknown which factors cause the initial constriction step during peroxisome division. However, it has been shown that this process can occur independently of Fis1p and DLP1, two components of the peroxisomal fission machinery (see Section 2.2.3) [200, 201]. In addition, it has been proposed that this process may be mediated by lipids. This hypothesis is based on the

observation that, in the yeast *Y. lipolytica*, peroxisome division is initiated by an acyl-CoA oxidase/Pex16p-regulated signaling cascade that originates inside the organelle and finally results in the accumulation of diacylglycerol—a cone-shaped membrane lipid known for its ability to induce strong membrane bending [202]—in the cytosolic leaflet of the peroxisomal membrane bilayer [203, 204]. Whether or not a similar mechanism is operative in other organisms remains to be investigated.

2.2.3. Fission Factors. Peroxisome fission is the best characterized step in the peroxisome division process. An obvious reason for this is that the protein machinery involved in this process is shared with mitochondria [205]. In all organisms studied so far, the final fission step of peroxisomes is mediated by dynamin-like proteins (e.g., Dnm1p in *H. polymorpha*, Dnm1p and Vps1p in *S. cerevisiae*, DRP3A in plants, and DLP1 in mammals) [206–210]. These proteins are large self-oligomerizing GTPases which can form a ring-like structure around their target membrane and constrict and sever this membrane in a GTP hydrolysis-dependent manner [211]. Importantly, to carry out their function, these predominantly cytosolic proteins need to be recruited to their target membrane [212]. This occurs through interaction with both soluble (e.g., Mdv1p in *H. polymorpha*, and Mdv1p and Caf4p in *S. cerevisiae*) and tail-anchored (e.g., Fis1p in *S. cerevisiae* and *H. polymorpha*; and Fis1p and Mff1 in mammals) adaptor proteins [192, 201, 210, 213, 214]. Cognate homologues of Mdv1p and Caf4p have not yet been found in higher eukaryotes [205]. How the tail-anchored adaptor proteins are recruited to the peroxisomal membrane and whether or not the peroxisomal and mitochondrial pools of these proteins can be exchanged is also not yet known. However, it has been shown that, in at least some organisms, these proteins can interact with members of the Pex11p family [215–218]. Finally, it should be mentioned that, in organisms in which multiple dynamin-like proteins or membrane-associated adaptor proteins have been associated with peroxisome fission, these proteins seem to be part of independent fission machineries [213, 214]. This finding suggests that these proteins may play distinct roles in the fission process. The observation that the division of peroxisomes appears to require Vps1p and Dnm1p in glucose- and oleate-grown cells of *S. cerevisiae*, respectively, is in line with this hypothesis [213].

2.3. Peroxisome Degradation. In order to regulate peroxisome function and restrain damage during cellular aging, superfluous and dysfunctional peroxisomes have to be selectively removed. Biochemical and genetic studies in different organisms have shown that peroxisome degradation can occur through at least three different mechanisms: macropexophagy, micropexophagy, and 15-lipoxygenase-mediated autolysis [219–221]. Macro- and micropexophagy (analogous to macro- and microautophagy) are two morphologically and mechanistically distinct types of peroxisome-specific vacuolar/lysosomal degradation pathways that utilize the core machinery of autophagy [222]: (i) during macropexophagy, individual peroxisomes are selectively

sequestered by a newly formed double-membrane vesicle, and these structures—called pexophagosomes (analogous to autophagosomes)—subsequently fuse with the vacuolar/lysosomal membrane; (ii) during micropexophagy, a cluster of peroxisomes is step-wisely surrounded by vacuolar/lysosomal membrane protrusions, which are then sealed through a process that involves a newly formed cup-shaped double-membrane structure, the micropexophagy-specific membrane apparatus (MIPA) [222, 223]. Both types of membrane engulfment finally result in the exposure of the incorporated peroxisome(s) to vacuolar/lysosomal hydrolases. Peroxisome autolysis is considered to be a nonselective process that is triggered by the insertion of 15-lipoxygenase, a lipid-peroxidizing enzyme, into the organellar membrane [224]. This causes focal membrane disruptions, which in turn are accompanied by content release into the cytosol. Albeit it is often thought that 15-lipoxygenase-mediated autolysis of peroxisomes (and other cell organelles) mainly occurs during differentiation of specific cell types such as reticulocytes and lens fiber cells, there is some evidence that this process may also be physiologically relevant in rat hepatocytes [220]. Nevertheless, despite this, the prevailing assumption is that the (selective) clearance of (superfluous) peroxisomes in most mammalian cell types is mainly—if not entirely—dependent on macropexophagy [225–228]. In other organisms, the mode of pexophagy may be different and can even depend on the metabolic state of the cell [229, 230].

To date, more than 35 AtTophagy-related (ATG) genes have been identified [221, 230]. The proteins encoded by these genes, collectively referred to as Atg proteins [231], are required for selective and nonselective autophagy pathways. Interestingly, all these pathways require a core molecular machinery, which is conserved from yeast to man [232]. However, selective degradation pathways such as pexophagy require additional components and mechanisms to recognize the organelles destined for turnover [232]. These adaptations are often species-specific [219]. The aim of this section is to provide an overview of our current knowledge on how superfluous or dysfunctional peroxisomes are recognized for autophagic sequestration. For a detailed description of the core molecular machinery involved in autophagosome formation, maturation, and degradation within lysosomes, I refer to other excellent reviews [233–236]. Possible peroxisome quality control mechanisms are discussed in Section 3.4.

2.3.1. Triggers for Degradation. A long standing and still open question in the field is how a cell recognizes superfluous and dysfunctional peroxisomes destined for autophagic degradation. Currently, two hypotheses have been proposed. The first one is that organelles having lost their protein import capacity are targeted for degradation [237], and the second one is that peroxisome degradation is initiated by a disturbance in intraperoxisomal redox balance [238]. The first hypothesis is mainly based on the observations that Pex3p and Pex14p, two peroxisomal membrane-bound peroxins essential for protein import (see Section 2.1.4), are also required for (macro)pexophagy (see Section 2.3.2). How

Pex3p and Pex14p can perform these apparently opposite functions is not yet well understood. However, it has been proposed that changes in the composition or activity of Pex3p- and/or Pex14p-containing protein import complexes may result in the exposure of a protein domain (e.g., the N-terminus of Pex14p), which in turn may be recognized by a yet unknown protein that tags the organelle for sequestration by the autophagosome [237, 239, 240]. The second hypothesis is based on the observation that blocking the autophagic degradation of peroxisomes in mammalian cells resulted in a gradual increase in the number of organelles with a disturbed redox balance [238]. The recent finding that excessive peroxisomal ROS formation triggers the degradation of these organelles in *H. polymorpha*, is in line with this hypothesis [241].

2.3.2. Recognition Factors. As already mentioned above (see Section 2.3.1), mounting evidence suggests that Pex3p and Pex14p also play a role in the initiation of macropexophagy. For Pex14p, it has been shown that its highly conserved N-terminal region is required for macropexophagy in *H. polymorpha* [239]. In addition, it has been reported that the *P. pastoris* and Chinese hamster orthologues of this protein can, respectively, bind to Atg30, the pexophagy receptor in methylotrophic yeasts (see Section 2.3.3), and LC3-II, an autophagosomal membrane marker [227, 242]. For Pex3p, the situation is more complex and conflicting data have been reported: in *H. polymorpha*, the protein needs to be removed from the peroxisomal membrane before macropexophagy can be initiated [243]; in *S. cerevisiae*, the protein functions as a peroxisomal docking factor for Atg36, the pexophagy receptor in budding yeasts (see Section 2.3.3) [230]; in *P. pastoris*, the peroxin participates in the recruitment and phosphorylation-dependent activation of Atg30 (see above) [242].

Finally, it has been shown that peroxisomes in mammalian cells can be specifically targeted for autophagic degradation by (mono)ubiquitination of surface-exposed domains of membrane proteins [244, 245]. Importantly, the observation that p62/SQSTM1—an adaptor protein between ubiquitinated substrates and the autophagic machinery (see Section 2.3.3)—is required for normal peroxisome turnover [244], strongly indicates that the regulation of basal peroxisome levels may involve the ubiquitination of endogenous proteins at the outside of the peroxisomal membrane. Unfortunately, no such proteins have yet been identified. A likely candidate may be Pex5p, the cycling PTS1 import receptor (see Section 2.1.2). Indeed, it is well known that this receptor needs to be (mono)ubiquitinated at the peroxisomal membrane (see Section 2.1.7) before it can be recycled back to the cytosol (see Section 2.1.8). In this context, it is attractive to hypothesize that, in case the receptor recycling process is disturbed, the accumulation of ubiquitinated Pex5p molecules at the peroxisomal membrane may serve as signal for peroxisome degradation [244]. Note that, if true, such a model would not only provide a direct molecular link between the protein import competence of individual peroxisomes and their susceptibility to degradation, but also explain why the highly conserved N-terminal domain of

Pex14p—which functions as docking site for Pex5p at the peroxisomal membrane (see Section 2.1.4)—is necessary for peroxisome degradation (see above).

2.3.3. Pexophagy Receptors. Every selective autophagy pathway studied to date requires the involvement of specific cargo receptors [221]. These receptors, which act alone or in combination with specific adaptor proteins, recognize their substrates and connect them with the core Atg machinery to allow their specific sequestration [246]. Currently, three “pexophagy receptors” have been identified: Atg30 (in *P. pastoris* and other methylotrophic yeasts), Atg36 (in *S. cerevisiae* and related yeasts), and p62/SQSTM1 (in mammals) [230, 242, 244]. Atg30 is necessary for the formation of pexophagy intermediates (e.g., the MIPA structure during micropexophagy, and the pexophagosome during macropexophagy) in *P. pastoris* [242]. It is recruited to peroxisomes by Pex3p and (phosphorylated) Pex14p, and—upon induction of pexophagy—the protein becomes phosphorylated by a hitherto unknown kinase. Phosphorylated Atg30 can in turn interact with other components of the autophagic machinery (e.g., Atg11 and Atg17) and localize transiently at the preautophagosomal structure. Atg36 is also recruited to peroxisomes in a Pex3p-dependent manner, and this pexophagy receptor brings the organelle into contact with the core Atg machinery via direct interaction with Atg11. Interestingly, despite their functional similarities, Atg36 and Atg30 do not display any sequence homology [230, 247].

Finally, it has been shown that the mammalian autophagy adaptor p62/SQSTM1 can mediate the selective autophagic degradation of ubiquitin-positive peroxisomes [244]. This protein can simultaneously interact with a ubiquitin-conjugated substrate (via a ubiquitin-binding domain) and the autophagosome-bound LC3-II (via an LC3-interacting region), allowing it to act as an adaptor between the substrate and the autophagic machinery [248]. Unlike Atg30 and Atg36, p62/SQSTM1 does not only function as a selective pexophagy receptor, but is also involved in other selective autophagy-related processes such as the degradation of ubiquitinated protein aggregates and the removal of dysfunctional ubiquitin-positive mitochondria [242]. In addition, it is not yet clear whether or not p62/SQSTM1 is the only pexophagy receptor in mammals. In fact, mammalian cells also contain other autophagy adaptors (e.g., NBR1 and NDP52) that have a similar domain structure to p62/SQSTM1, and it remains to be tested if these proteins are also involved in the selective degradation of peroxisomes [248, 249].

3. Mechanisms

As already pointed out in the introduction, peroxisomes are highly versatile organelles which continuously adapt to prevailing environmental conditions. This implies that cells need mechanisms to rapidly adjust the number of these organelles upon changes in their cell cycle or in response to various stimuli. These mechanisms, which underlie the process of peroxisome dynamics, have only recently begun to be uncovered. This section is intended to provide more information on how new peroxisomes arise and how the vitality

and abundance of these organelles are regulated by quality control mechanisms. The main focus of attention is given to the formation of the peroxisomal membrane, the different modes of peroxisome multiplication, the regulatory aspects of peroxisomal protein import, the organelle's quality control systems, and the potential key regulators of peroxisome homeostasis.

3.1. Formation of the Peroxisomal Membrane. The assembly of functional peroxisomal membranes requires the coordinated synthesis and uptake of both lipids and proteins. Over the years, growing evidence has been collected that this process is strongly dependent on the endoplasmic reticulum. For example, peroxisomes are not capable of synthesizing their own membrane phospholipids [250], and—at the moment—there are indications that the phospholipids required for peroxisomal membrane expansion can be acquired from the ER by vesicular (e.g., in *P. pastoris*) and nonvesicular (e.g., in mammals) transport pathways [251, 252]. In addition, there is ample but conflicting evidence that also PMPs originate their life in the ER (see Section 2.1.1) [41, 43, 253, 254]. If true, these findings suggest that the origin and maintenance of peroxisomes involves a *de novo* pathway from the ER (see Section 3.2). However, this is a controversial issue that remains subject to debate. Indeed, there is also a wealth of information to support the view that newly synthesized PMPs insert directly from the cytosol into the membrane of preexisting organelles (see Section 2.1.1) [42, 142, 255, 256]. If these findings are correct, they suggest that peroxisomes multiply by growth and division of preexisting organelles (see Section 3.2). Importantly, it is not the purpose of this paper to rehearse all the arguments presented in these studies or to settle the dispute about whether new peroxisomes arise *de novo* from the ER or by fission of preexisting organelles. Both assembly pathways may operate simultaneously or serve different purposes in different organisms and/or conditions. The working models for each of these pathways will be discussed in the following section.

3.2. Different Models of Peroxisome Multiplication. In the current model of how new peroxisomes multiply by *de novo* synthesis from the ER, (a select set of) PMPs are initially inserted into the ER via the Sec61p translocon or the GET complex (see Section 2.1.5). Next, these proteins are sorted and sequestered into specialized Pex3p-containing ER exit sites, which—in mammals—are characterized by the presence of Sec16B, a peripheral membrane protein involved in COPII vesicle formation [257]. Finally, the PMP-containing preperoxisomal vesicles bud from the ER in an ATP-, temperature-, cytosol-, and Pex19p-dependent manner [43, 108, 170, 258], and subsequently mature into functional peroxisomes through a sequence of steps that most likely require (i) Pex1p- and Pex6p-dependent homotypic membrane fusions [180, 259], and (ii) the insertion of other PMPs that enable subsequent matrix protein import [260]. Note that the molecular players involved in these processes may differ among species. For example, Pex16p is essential to recruit other PMPs to the ER in mammalian

cells [41]. However, this peroxin is not necessary for *de novo* peroxisome biogenesis in *Y. lipolytica* [261] and *P. chrysogenum* [262], and the protein is even absent in members of the Saccharomycetaceae family [119]. On the other hand, Pex25p—a protein absent in many organisms, including mammals—is essential to generate peroxisomes *de novo* in *S. cerevisiae* and *H. polymorpha* mutants lacking these organelles [193, 263]. Finally, it is worthwhile mentioning that the artificial targeting of Pex3p to the mitochondrial membrane can initiate a *de novo* synthesis of peroxisomes in *S. cerevisiae* [264]. This observation suggests that the targeting of Pex3p to any endomembrane may initiate peroxisome formation, a hypothesis in line with previous work showing that (i) Pex3p-mediated peroxisome biogenesis may occur independently of COPI- and COPII-dependent membrane traffic [265], (ii) inactivation of Sec61p does not affect peroxisome biogenesis [146], and (iii) peroxisomes can also multiply by growth and division (see next paragraph).

According to the classical growth and division model, peroxisomes are semiautonomous cell organelles that increase in size by the posttranslational import of newly synthesized peroxisomal matrix and membrane proteins and multiply by the division of preexisting organelles [37]. From this point of view, the ER is considered to be only a source of membrane phospholipids for organelle-surface enlargement. This lipid transfer may occur through specific vesicular transport mechanisms [252] or close membrane associations [251], which can be frequently observed between peroxisomes and smooth segments of the ER [266]. Over the last years, it has become clear that peroxisome division is a complex multistep process in which the matrix protein content is unevenly distributed over the resulting daughter organelles [192, 210, 228, 256]. A successful completion of this process includes (i) the Pex11p β -mediated formation of a peroxisomal subdomain at one side of a preexisting peroxisomes, (ii) the extension of this domain and the formation of a Pex3p-, Pex16p-, and Pex19p-positive PMP-import competent membrane compartment, (iii) the constriction of this compartment (see Section 2.2.2), (iv) the assembly and activation of the complete import machinery and the subsequent recruitment of PMPs and matrix proteins, and (v) the final fission into spherical peroxisomes (see Section 2.2.3) [256]. This model raises two interesting questions. First, which factors coordinate the nonrandom distribution of matrix and membrane proteins within the globular or tubular membrane domains? Second, what is the biological significance of asymmetric fission? Regarding the first question, one may predict that protein-protein interactions, active transport, cytoskeleton interactions, and/or membrane lipid microdomains may be involved [267]. In this context, it is interesting to mention that peroxisomes in *Y. lipolytica* contain dynamic ergosterol- and ceramide-rich domains which function as an organizing platform for the fusion of immature peroxisomal vesicles (see above) [268], and that lipid microdomains are also essential for peroxisome biogenesis in rat hepatocytes and HepG2 cells [269, 270]. Regarding the second question, it is tempting to speculate that asymmetric peroxisome fission may represent a quality control mechanism (see Section 3.4).

3.3. Regulatory Aspects of Peroxisomal Protein Import. To cope with changing environmental conditions, cells have evolved mechanisms to control the localization of numerous proteins. The list of proteins showing a (transient) dual localization to both peroxisomes and another subcellular compartment (e.g., mitochondria, the ER, and the cytosol) is continuously growing. The *cis*-acting molecular mechanisms underlying the dual targeting of individual “peroxisomal” proteins can be coupled to alternative splicing, the use of different translation initiation sites, proteolytic cleavage, and protein phosphorylation. For more specific information on each of these topics, I refer to another comprehensive review [271]. Here, I will address some aspects of how the peroxisomal matrix protein import apparatus can adapt to the heterogeneity of cargo molecules and how the activity of this machinery may be regulated in response to shifts in metabolic state.

As already discussed above (see Section 2.1.1), there is plenty of evidence showing that the peroxisomal matrix protein translocation apparatus is highly dynamic and flexible and can accommodate various substrates of different sizes. A long-standing question in the field is how this machinery can accommodate the transport of these substrates without compromising the permeability barrier of the peroxisomal membrane. Substantial progress was achieved when it was realized that Pex5p and Pex14p constitute the key components of the translocation pore, and that this channel can expand in diameter when cytosolic Pex5p-cargo complexes are encountered (see Section 2.1.5). To grasp the flexibility and dynamic nature of these Pex14p-Pex5p-PTS1 complexes, it is important to keep the following in mind: Pex5p and Pex14p both undergo substantial conformational changes upon binding to their interaction partners [86, 87, 272]; Pex14p molecules can dynamically assemble into high molecular mass homomeric complexes that preferentially bind cargo-bound Pex5p (cargo-unloaded Pex5p apparently disassembles these complexes) [84, 272, 273]; the N-terminus half of Pex5p is an intrinsically disordered domain that has been suggested to acquire rigidity upon interaction with Pex14p, thereby providing a possible lever-like mechanism for protein translocation across the organellar membrane [274]. Note that the latter idea is in line with the recent observation that the export of *S. cerevisiae* Pex18p, a protein whose N-terminus displays functional similarities with the N-terminus of Pex5p (see Section 2.1.3), is mechanically linked to the import of cargo-loaded Pex7p [128]. However, in this context, it is important to point out that there is currently no consensus on when cargo translocation precisely occurs; in mammals, Pex5p-mediated cargo-translocation seems to occur prior to Pex5p ubiquitination [153]; in yeast, Pex18p-mediated cargo-translocation seems to be directly linked to the cysteine-dependent ubiquitination of Pex18p [128].

At present, there is growing evidence that eukaryotic cells can adapt their intracellular protein distribution pattern in response to nutrient availability and cellular needs [275]. As (i) cellular metabolism is inherently linked to the intracellular redox status, and (ii) the intracellular localization (and activity) of many proteins is reversibly controlled by the

oxidation status of specific thiol-containing residues [276], it may not come as a surprise that also the localization of some “peroxisomal” proteins is affected upon changes in the cellular redox state. For example, it has been shown that the import of catalase is gradually impaired during the aging process (i.e., when the redox status is increasing) in mammalian cells [277], and that this import defect can be restored upon reestablishment of the cellular redox balance [238, 278]. In addition, it has recently been reported that the peroxisomal import of plant G6PD1—a catalytically active isoform of glucose-6-phosphate dehydrogenase (G6PD)—involves a cysteine-dependent interaction with cytosolically localized G6DP4—a, “catalytically inactive” form of G6DP [279]. Note that, although these data are highly suggestive, one can currently not exclude that the cytosolic localization of both catalase and G6PD1 is a direct or indirect result of reduced energy availability. Indeed, it is well known that the import of proteins into the peroxisomal matrix is directly coupled to the ATP-dependent recycling of the ubiquitinated import receptor (see Sections 2.1.7 and 2.1.8) [157].

3.4. Organelle Quality Control. Peroxisomes house many enzymes that produce reactive oxygen species (ROS) as part of their normal catalytic cycle [14]. As such, peroxisome maintenance and turnover is of great importance for cellular homeostasis and survival. Currently, three peroxisomal quality control mechanisms have been identified.

First, peroxisomes in many types of eukaryotic cells contain a peroxisomal variant of the ATP-stimulated Lon protease, an enzyme thought to be involved in the proteolysis of oxidized proteins [280, 281]. Recently, it has been shown that the *P. chrysogenum* orthologue of this protein, called Pln, can degrade oxidized proteins *in vitro*, and that a deficiency in Pln activity is associated with the formation of protein aggregates in the peroxisomal matrix and enhanced oxidative stress [282]. These observations allowed the authors to speculate that peroxisomal Lon proteases actively assist in the peroxisomal matrix protein quality control process, and—in case these proteases do not function properly—this may result in the accumulation of damaged and nonassembled protein aggregates within the matrix (e.g., catalase; and this in turn may lead to an imbalance in H₂O₂ production and degradation). However, in this context, it is necessary to mention that (i) some organisms (e.g., *S. cerevisiae* and *D. melanogaster*) lack a peroxisomal Lon variant [283], and (ii) in other organisms (e.g., mammals), the peroxisomal Lon-protease is believed to be involved in peroxisomal matrix protein import [283, 284]. Note that it cannot be excluded that peroxisomal Lon acts as a multifunctional protein.

Second, it has been observed that, in cultured mammalian cells, the turnover rates of some PMPs (e.g., Pex3p: $t_{1/2}$ = 2–6 h) are much faster than that of matrix proteins ($t_{1/2}$ = \pm 2 days) [77, 228], and that the half-life of these PMPs can be extended by inhibiting the proteasome degradation pathway [228]. These findings suggest that cells possess a mechanism to rapidly remove unwanted proteins from the peroxisomal membrane. However, the molecular mechanisms of how these proteins are tagged for degradation

and subsequently extracted from the membrane remain to be fully elucidated.

Third, it is well documented that a cell can remove whole peroxisomes by a process called pexophagy (see Section 2.3). As already discussed in Section 2.3.1, it is not precisely known how a cell recognizes peroxisomes destined for autophagic degradation. In addition, as peroxisomes—unlike mitochondria [285]—do not contain a fusion machinery which allows them to mix, segregate, and eliminate damaged components from the functional networking population [228, 286], these organelles require other mechanisms to prevent the degradation of freshly imported (and therefore most likely functional) proteins. One such mechanism may be nonsymmetric fission (see Section 3.2), a process that would allow the organelle to retain dysfunctional proteins within the mother organelle, which—after a limited number of fission events—is targeted for autophagic degradation [210, 228]. Again, a burning question is how a cell can distinguish “dysfunctional mother organelles” from “functional daughter organelles.” The answer may lie in the observation that the PTS-receptor docking complex (see Section 2.1.4) and the PTS-receptor ubiquitination machinery (see Section 2.1.7) are physically connected by a linker protein (e.g., Pex3p in *P. pastoris*, and Pex8p in *S. cerevisiae*) [240, 287]. Indeed, as the accumulation of dysfunctional proteins in the mother organelles may cause local oxidative stress [238, 282], it is tempting to speculate that severe stress conditions may result in the dissociation of the two subcomplexes and the subsequent exposure of pexophagy receptor-recognition sites (see Section 2.3.2).

3.5. Key Regulators of Peroxisome Homeostasis. A tight regulation of peroxisome dynamics and function in response to environmental conditions is essential for normal development and cellular homeostasis (see Section 4). The process of peroxisome homeostasis itself relies on the balanced activity of organelle biogenesis, inheritance, and degradation. This section mainly focuses on the potential key regulators involved in peroxisome formation and removal. For factors and mechanisms involved in peroxisome partitioning during the cell cycle, I refer to another informative review [288].

An increasing amount of evidence suggests that the processes of peroxisome development and turnover converge at the peroxins Pex3p and Pex14p (see Sections 2.1.4 and 2.3.2), indicating that these peroxins may function as central regulators of peroxisome homeostasis [230, 239]. A central and open question is how Pex3p and Pex14p can serve apparently opposite functions at different stages of the organelle's life cycle. One potential answer to this question may come from the observations that Pex14p can be phosphorylated *in vivo*, and that the nonphosphorylated and phosphorylated isoforms of this peroxin are involved in peroxisome biogenesis and macropexophagy, respectively [289]. However, the molecular mechanisms regulating Pex14p phosphorylation *in vivo* remain to be clarified.

Interestingly, there is also strong evidence that the reversible phosphorylation of other proteins (or even lipids) may play an important role in peroxisome homeostasis. For example, it is known that the pexophagy receptor Atg30 is

activated at the peroxisomal membrane through phosphorylation by a not yet identified kinase (see Section 2.3.3). In addition, it has been reported that, in *S. cerevisiae* and in *P. pastoris*, Pex11p can be reversibly phosphorylated in response to nutritional cues, and that the phosphorylated protein can recruit Fis1p to the peroxisomal membrane to promote peroxisome division [218, 290]. Furthermore, it has been shown that the Sltp2 mitogen-activated protein kinase signal transduction pathway is necessary for pexophagy in *S. cerevisiae* [249]. Finally, it should be noted that the *H. polymorpha* and *S. cerevisiae* orthologues of Vps34p, a phosphatidylinositol 3-kinase responsible for the synthesis of phosphatidylinositol 3-phosphate, are also required for pexophagy [291, 292]. A major challenge for the future will be to integrate these observations into a wider and coherent framework.

4. Functions and Dysfunctions

Peroxisomes play an indispensable role in cellular lipid metabolism [18]. In addition, there is growing evidence that these organelles actively contribute to the maintenance of the cellular redox balance [14]. Unwanted alterations in peroxisome function may invoke serious consequences for affected organisms [25, 293, 294]. However, these consequences may vary depending on the organism, the type of defect, and the environment. For example, genomic mutations inactivating *PEX* genes may have the potential to exhibit no visible phenotype (e.g., in yeast cells grown on glucose medium) or to cause a debilitating or even fatal condition (e.g., in human beings, see Section 1). Importantly, peroxisomes closely cooperate with other cellular compartments to carry out their physiological functions. At the morphological level, this is perhaps best illustrated by the observation that these organelles display extensive contact sites with the ER [266], lipid droplets [295], and mitochondria [296], and it has been proposed that these contact sites may facilitate the transfer of metabolites [30, 251]. At the functional level, this is nicely exemplified by the finding that peroxisomes and mitochondria share the same fission machinery (see Section 2.2.3), a mechanism allowing the cell to fine-tune peroxisomal and mitochondrial metabolism [28]. The importance of this process is also reflected in the observation that a dominant-negative point mutation in DLP1 (see Section 2.2.3) is fatal in humans [297].

As reviewed elsewhere, strong arguments have been presented that peroxisomal metabolism and cellular aging are closely intertwined [298, 299]. In addition, there is a substantial body of evidence linking peroxisomal dysfunction to the initiation and progression of age-related diseases, such as type 2 diabetes, cancer, and some neurodegenerative disorders like Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Fransen et al., manuscript submitted). The molecular mechanisms underlying such events are just beginning to be unraveled. A hypothesis gaining popularity is that a disturbance in peroxisome function initiates signaling events that ultimately result in mitochondrial alterations which, in turn, trigger the activation of mitochondrial stress pathways [238, 298]. However, further

work is needed to elucidate how peroxisomes are incorporated into such intracellular communication networks, and how changes in mitochondrial metabolism may influence peroxisome dynamics. Finally, additional research is required to better understand how lysosomal dysfunction may lead to defects in peroxisome turnover. In this context, it would be particularly interesting to know to what extent the complex phenotypes of various lysosomal storage disorders may reflect impairment in peroxisome function.

5. Summary and Outlook

Over the last decades, remarkable progress has been made in our understanding of how peroxisomes are formed and degraded within cells. General key findings include the observations that the peroxisome biogenesis and selective degradation pathways converge on a select set of proteins (*in casu* Pex3p and Pex14p), and that functional domains—rather than entire proteins—are conserved throughout evolution. However, despite this, there is still a large gap in our knowledge of how disturbances in peroxisome homeostasis may affect the health and viability of an organism. For example, it is not yet known if, how, and to what extent defects in pexophagy can lead to human disease. Another challenging and open question is whether or not subtle differences in any of the functionally conserved peroxin domains can represent an interesting therapeutic target to treat human diseases (e.g., the bloodstream form of *T. brucei*, a human parasite causing sleeping sickness, relies entirely on glycosomal metabolism for the generation of energy; see Section 1). Finally, it remains to be investigated how changes in peroxisome dynamics affect cellular metabolism, signaling and stress response. Gaining a better insight into these complex interactions is pivotal for a coherent understanding of how these organelles function in health and disease.

Abbreviations

AAA-ATPase:	ATPase associated with a various cellular activities
ATG :	Autophagy-related gene
Atg:	Autophagy-related protein
DLP:	Dynamin-like protein
dysferlin:	Dystrophy-associated fer-1-like protein
ER:	Endoplasmic reticulum
G6PD:	Glucose-6-phosphate dehydrogenase
i-PTS:	Internal peroxisomal matrix protein targeting signal
MIPA:	Micropexophagy-specific membrane apparatus
mPTS:	Peroxisomal membrane protein targeting signal
OMIM:	Online Mendelian inheritance in man
PBD:	Peroxisome biogenesis disorder
PED:	Peroxisomal enzyme deficiency
PEX :	Gene encoding peroxin
Pexp:	Peroxin
PMP:	Peroxisomal membrane protein
PTS1:	C-terminal peroxisomal targeting signal

PTS2: N-terminal peroxisomal targeting signal
 RING: Really interesting new gene
 SH3: Src-homology region 3
 TPR: Tetratricopeptide repeat
 WD40: Tryptophan-aspartic acid repeat domain of approximately 40 amino acid residues.

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