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

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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 (H2O2) [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 βlactam 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.

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.

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 "Pexp", 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.

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 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].

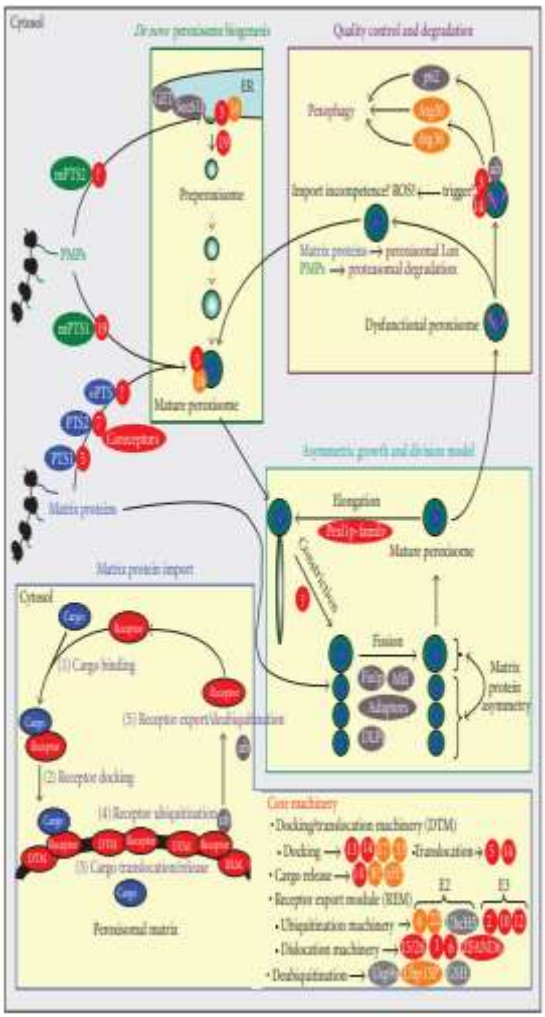

Figure 1: Model of peroxisome dynamics. New peroxisomes can be formed de novo from the ER or by asymmetric growth and division of preexisting organelles. In the de novo pathway, a select set of PMPs is inserted into the ER via the Sec61p translocon (or the GET complex) and sequestered into specialized 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.

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 I PMPs (1 transmembrane segment, Nmatrix-Ccytoplasm) and near the C-terminus in type 2 PMPs (1 transmembrane segment, Ncytoplasm-Cmatrix) [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 membranespanning 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.

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 finetune

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.

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.

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