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Minireview

The peroxisomal protein import machinery

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Abstract Peroxisomes are unique organelles whose physiological functions vary depending on the cellular environment or metabolic and developmental state of the organism. These changes in enzyme content are accomplished by the dynamically operating membrane and matrix protein import machineries of peroxisomes that rely on the concerted function of at least 20 peroxins. The import of folded matrix proteins is mediated by cycling receptors that shuttle between the cytosol and peroxisomal lumen. Receptor release back to the cytosol represents the ATP-dependent step of peroxisomal matrix protein import, which consists of two energy-consuming reactions: receptor ubiquitination and dislocation.

Keywords: Peroxisome biogenesis; Protein targeting; PEX; Peroxin; Ubiquitination; AAA ATPases

1. Introduction

Peroxisomes are single-membrane-bound organelles that fulfil a large variety of functions in virtually all eukaryotic cells. They exhibit a pronounced morphological and metabolic plasticity, which is dependent on the organism, cell type and prevailing environmental conditions.

Peroxisomes were initially described as ''microbodies'' in electron microscopic pictures of mouse kidney cells [1]. Subsequently, de Duve developed the Nobel Prize honoured differential and gradient centrifugation method for cell-fractionation [2]. These tools were instrumental for the isolation of an organelle fraction containing catalase and hydrogen peroxide producing oxidases which was named peroxisomes [3].

Peroxisomes appear to own a unique variability in enzyme content and thus metabolic function, which mark them as ''multi-purpose organelles'' that are adjusted according to the cells needs [4]. The enzymes in the peroxisomal matrix are linked to different biochemical pathways. A central function is the β-oxidation of fatty acids and the detoxification of the thereby produced hydrogen peroxide. This pathway is exclusively localized in the peroxisomal compartment of fungi and plants, whereas in mammalian cells the breakdown of different types of fatty acids is distributed between peroxisomes and mitochondria [5]. The decomposition of fatty acids mark peroxisomes as one source of signalling molecules such as reactive oxygene species (ROS) in plants [6] or lipid ligands for the peroxisome proliferator-activated receptors (PPAR) family in humans, whose dysfunction is linked to hepatocellular carcinoma [7]. The enzymes of the peroxisomal β-oxidation pathway are also involved in the synthesis of chemical compounds which function as phytohormones in plants, such as jasmonates or indol-3-acetic acid [6]. Other functions include α-oxidation of branched-chain fatty acids in mammals and plants [8], the main reactions of photorespiration in leaf peroxisomes [9], the final steps of penicillin biosynthesis in some filamentous fungi [10], or synthesis of bile acid and ether lipids such as plasmalogens, which contribute more than 80% of the phospholipid content of the white matter in the brain [11]. The generation and detoxification of hydrogen peroxide and other ROS relates peroxisomes to the molecular process of aging [12].

Specialized forms of peroxisomes were initially described as separate organelles. In this context, the glyoxysomes of plant seedlings and some fungi house enzymes of the glyoxylate cycle that enable the conversion of lipids into carbohydrates [13]. Filamentous fungi contain Woronin-bodies additionally to other microbodies. Their task is to seal septal pores in their hyphae in order to prevent fatal cytosolic bleeding [14,15]. The glycosomes of trypanosomes harbour key enzymes of glycolysis [16] which might also be true for the microbodies of the pathogenic basidiomycete *Cryptococcus neoformans* [17].

The study of peroxisomal biogenesis and protein import was hampered for a long time by their great fragility and low abundance in many tissues. This situation changed when it was discovered that peroxisome proliferation can be induced in bakers yeast by manipulation of the carbon source [18]. When *S. cerevisiae* cells were grown on oleic acid as the sole carbon source, peroxisomes become essential for growth because they represent the exclusive site for fatty acid degradation in yeast. This allowed the screening of mutants affected in the biogenesis of peroxisomes, referred to as *pex* mutants [19,20] with *PEX* being the acronym for the corresponding gene, the gene products were collectively named peroxins [21]. To date 32 peroxins are known [22,23]. They are involved in the three key stages of peroxisomal development: (i) formation of the peroxisomal membrane, (ii) peroxisome proliferation and (iii) compartmentalization of peroxisomal matrix proteins.

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Abbreviations: AAA, ATPase associated with various cellular activities; ERAD, endoplasmatic reticulum associated degradation; PTS, peroxisomal targeting signal; RING, really interesting new gene; Ub, ubiquitin

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2. Peroxisome formation and inheritance

The origin of the peroxisomal membrane has been a matter of debate for a long time. Early studies which were based on ultrastructural investigations using electron microscopy and suggested that peroxisomes generate by budding from the endoplasmic reticulum (ER) [24]. Later, biochemical data demonstrated that peroxisomal matrix proteins are synthesized on free ribosomes in the cytosol and that these proteins are imported posttranslationally in pre-existing peroxisomes. Based on these results, an ER-independent model, termed "growth and division model", was proposed, which predicted that peroxisomes multiply autonomously like mitochondria or chloroplasts [25]. Although the sum of the published data placed peroxisomes among these autonomously multiplying organelles, the observation that the reintroduction of a gene into a peroxisome-lacking deletion strain, could induce a de novo formation of peroxisomes, remained difficult to explain. Recent studies, mainly based on real-time fluorescence microscopy combined with biochemical approaches, provided evidence for the ER being the source for the origin of peroxisomal membranes, at least during de novo formation [23,26]. This process requires the integral membrane protein Pex3p, which is localized to the ER at first, concentrates in foci that bud off in a Pex19p-dependent manner and mature to functional peroxisomes [26]. Peroxisome formation in mammalian cells also depends on the function of cotranslationally inserted Pex16p [27]. Little is known about the ER-targeting of Pex3p to the ER as well as the budding and subsequent maturation of peroxisomes. In *Yarrowia lipolytica*, this process involves formation and fusion of pre-peroxisomal vesicles which is thought to depend on the ATPase associated with various cellular activities (AAA) proteins Pex1p and Pex6p [28].

Based on data from plant and mammalian cells, a retrograde pathway of membrane portions from the peroxisome to the ER is discussed as well [29,30]. As a consequence of the recent results, peroxisomes are believed to constitute a semi-autonomous part of the secretory pathway [26] (Fig. 1).

However, new peroxisomes are believed to arise primarily by duplication of the pre-existing peroxisomes. To this end, peroxisomes contain an elaborate fission and proliferation machinery. Pex11p was among the first components of this device which were discovered. The deletion of *PEX11* leads to a strong reduction of peroxisome number together with an increase in size of the remaining peroxisomes. Additionally, the Pex11p-type peroxins Pex25p and Pex27p play a role in controlling size and number of *S. cerevisiae* peroxisomes. Their function is thought to induce constriction of the organelle [31] (Fig. 1). Pex25p is also described as recruitment factor for the GTPase Rho1p [32] which controls actin reorganisation at the peroxisomal membrane and thus may be required for the peroxisomal division and the inheritance process. The scission

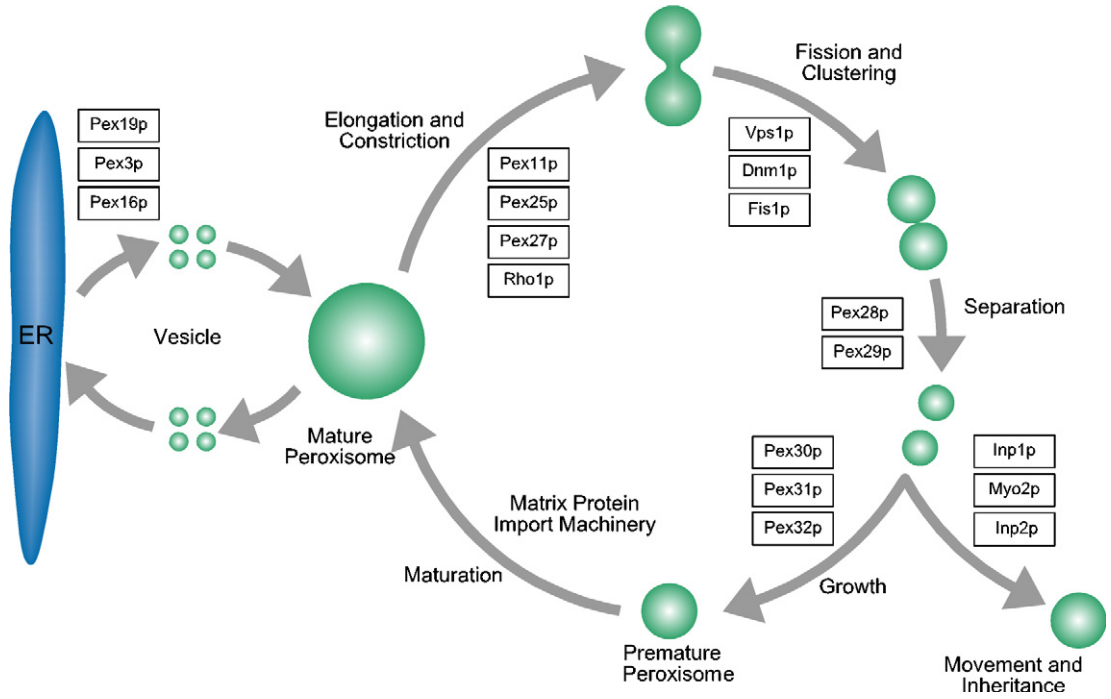

Fig. 1. Model for the division and proliferation of peroxisomes. Pex19p, Pex3p and Pex16p are required for the formation of the peroxisomal membrane as they facilitate the insertion of peroxisomal membrane proteins. This explains their initial requirement for the de novo formation of peroxisomes at the ER. The formation of mature peroxisomes may involve the fusion of precursor vesicles, as observed in *Y. lipolytica*. The peroxisomal membrane proteins Pex11p, Pex25p and Pex27p are involved in the elongation and constriction of mature peroxisome. The GTPase Rho1p might be involved in this process because it binds to Pex25p. Consecutively, the peroxisome undergoes a fission event, which requires the dynamin-related proteins Vps1p(DLP1) and Dnm1p. The former is anchored to the peroxisomal membrane via Fis1p. The divided but clustered peroxisomes are supposed to be separated by Pex28p and Pex29p, which are the orthologues of *Y. lipolytica* Pex24p. The size of peroxisomes is thought to be regulated by Pex30p, Pex31p and Pex32p, which are the orthologues of *Y. lipolytica* Pex23p. The maturation process of the peroxisome is facilitated by the import of matrix proteins. Inheritance of peroxisomes requires Inp1p, Inp2p and Myo2p. The nomenclature refers to the proteins from *S. cerevisiae*, with the exception of Pex16p, which has not yet been identified in bakers yeast but is known to exist in humans, plants and other fungi.