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Minireview

MINIREVIEW

Yeast peroxisomes: structure, functions and biotechnological opportunities

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One sentence summary: Review describing properties of peroxisomes, including their biogenesis, inheritance, degradation, metabolic functions and potential use for biotechnological purposes.

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ABSTRACT

Peroxisomes are ubiquitous organelles found in most eukaryotic cells. In yeasts, peroxisomes play important roles in cell metabolism, especially in different catabolic processes including fatty acid β-oxidation, the glyoxylic shunt and methanol metabolism, as well as some biosynthetic processes. In addition, peroxisomes are the compartment in which oxidases and catalase are localized. New peroxisomes mainly arise by fission of pre-existing ones, although they can also be formed from the endoplasmic reticulum (ER). Peroxisomes consist of matrix-soluble proteins and membrane proteins known as peroxins. A total of 34 *PEX* peroxin genes and proteins have been identified to date. and their functions have been elucidated. Protein import into peroxisomes depends on peroxins and requires specific signals in the structure of transported proteins: PTS1, PTS2 and mPTS. The mechanisms of metabolite penetration into peroxisomes are still poorly understood. Peroxisome number and the volume occupied by these organelles are tightly regulated. Methanol, fatty acids and methylamine act as efficient peroxisome proliferators, whereas glucose and ethanol induce peroxisome autophagic degradation (pexophagy). To date, 42 Atg proteins involved in pexophagy are known. Catabolism and alcoholic fermentation of the major pentose sugar, xylose, depend on peroxisomal enzymes. Overexpression of peroxisomal transketolase and transaldolase activates xylose fermentation. Peroxisomes could be useful as target organelles for overexpression of foreign toxic proteins.

Keywords: peroxisomal enzymes; peroxins; peroxisome targeting; peroxisome propagation; pexopahgy; xylose alcoholic fermentation

OCCURRENCE AND GENERAL PROPERTIES OF PEROXISOMES IN DIFFERENT GROUPS OF EUKARYOTES

Peroxisomes are DNA-free organelles present in practically all eukaryotic cells. The only known exceptions are the protists *Giardia* and *Entamoeba*, and the fungi *Microsporidia*, which also do not contain mitochondria, and the mitochondria-containing protists belonging to *Apicomplexa* (Cavalier-Smith 1987; Schlüter *et al.* 2006; Gabaldón 2010). Peroxisomes could be defined as organelles that contain hydrogen peroxide-generating oxidases and the hydrogen peroxide-detoxifying enzyme catalase. Fatty acid β-oxidation is an important functional characteristic of most eukaryotic peroxisomes from yeast to humans. In animals, peroxisomes are additionally involved in oxidative degradation of long-chain fatty acids, purines, certain amino acids and pipecolynic acid, and in the synthesis of cholesterol, bile acids and ether lipids such as plasmalogens (Olivier and

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Krisans 2000; Schrader and Fahimi 2008; Schrader *et al.* 2013; Faust and Kovacs 2014). Plant peroxisomes are classified into three groups: glyoxysomes, leaf peroxisomes and unspecialized peroxisomes. They all contain catalase; however, they differ strikingly in other features. Glyoxysomes are involved predominantly in the glyoxylate cycle and fatty acid β-oxidation, while leaf peroxisomes play a role in metabolizing glycolic acid. The glyoxylic acid cycle is also localized in yeast peroxisomes. There are ~50 proteins in animal and fungal peroxisomes, and ~100 proteins in plant peroxisomes (Michels *et al.* 2005). In *Saccharomyces cerevisiae*, there are at least 66 peroxisomal proteins (Kohlwein, Veenhuis and van der Klei 2013). Proteomic and genetic studies continuously reveal new functions for peroxisomes. In some mycelial fungi, there are specialized peroxisomes known as Woronin bodies derived by budding from common peroxisomes and used to plug septal pores in the hyphae during injury (Schrader and Fahimi 2008). Several reactions of penicillin biosynthesis in fungi are localized in peroxisomes (Bartoszewska *et al.* 2011). In plants and mycelial fungi, one reaction of biotin biosynthesis is located in peroxisomes (Magliano *et al.* 2011; Tanabe *et al.* 2011) and it could be hypothesized that the same is true for biotin-prototrophic yeasts (Maruyama and Kitamoto 2013). In some parasitic protists (*Kinetoplastida*), peroxisomes contain the main reactions of glycolysis and are known as glycosomes (Moyersoen *et al.* 2004). A deficiency in peroxisomes leads to incurable human diseases, such as Zellweger syndrome. Cells from patients with this syndrome were found to lack normal peroxisomes and contain only their rudiments devoid of several matrix proteins (Wanders and Waterham 2006). Typically, peroxisomes are quite small organelles, between 0.1 and 0.2 μm in size and surrounded by a single membrane; however, in methylotrophic yeasts, their size could be much larger (up to 1.5 μm; see Fig. 1) (Michels *et al.* 2005; van der Klei *et al.* 2006; Nagotu, Veenhuis and van der Klei 2010).

ROLE OF PEROXISOMES IN METABOLISM OF THE YEAST CELL

Biogenesis and degradation of peroxisomes and the role of this organelle in metabolism have been studied in many yeast species. The most popular species are baker's yeast *S. cerevisiae*, methylotrophic yeasts (*Pichia pastoris* and *Hansenula polymorpha*) and *Yarrowia lipolytica*. Methylotrophic yeasts are very convenient model organisms as they allow easy, fast and effective induction of massive proliferation and degradation of peroxisomes (van der Klei *et al.* 2006). It is interesting to note that the yeast peroxisome is the densest organelle with the highest protein concentrations among all organelles. The high protein concentration in these organelles often results in the formation of protein crystalloids in the peroxisomal matrix of methylotrophic yeasts, but not in *S. cerevisiae* (Kohlwein, Veenhuis and van der Klei 2013).

In yeasts, peroxisomes are involved in numerous metabolic reactions. Similarly to other organisms, yeast peroxisomes contain H_2O_2-producing oxidases (mostly containing FAD as coenzyme) and catalase that degrades hydrogen peroxide. It is assumed that in yeast, the fatty acid β-oxidation pathway and glyoxylic acid cycle reside in peroxisomes. Peroxisomes contain four main enzymes of β-oxidation (medium chain fatty acid CoA activation, oxidation, hydratation/dehydrogenation and thiolytical cleavage) as well as auxiliary peroxisomal enzymes involved in oxidation of odd-numbered and unsaturated fatty acids (van Roermund *et al.* 2003). The localization of glyoxylic acid cycle enzymes varies in different yeasts. For example, in *S. cerevisiae*, isocitrate lyase is a cytosolic enzyme, whereas in most other species this enzyme is localized in peroxisomes (Kunze *et al.* 2006). Recent findings demonstrate that

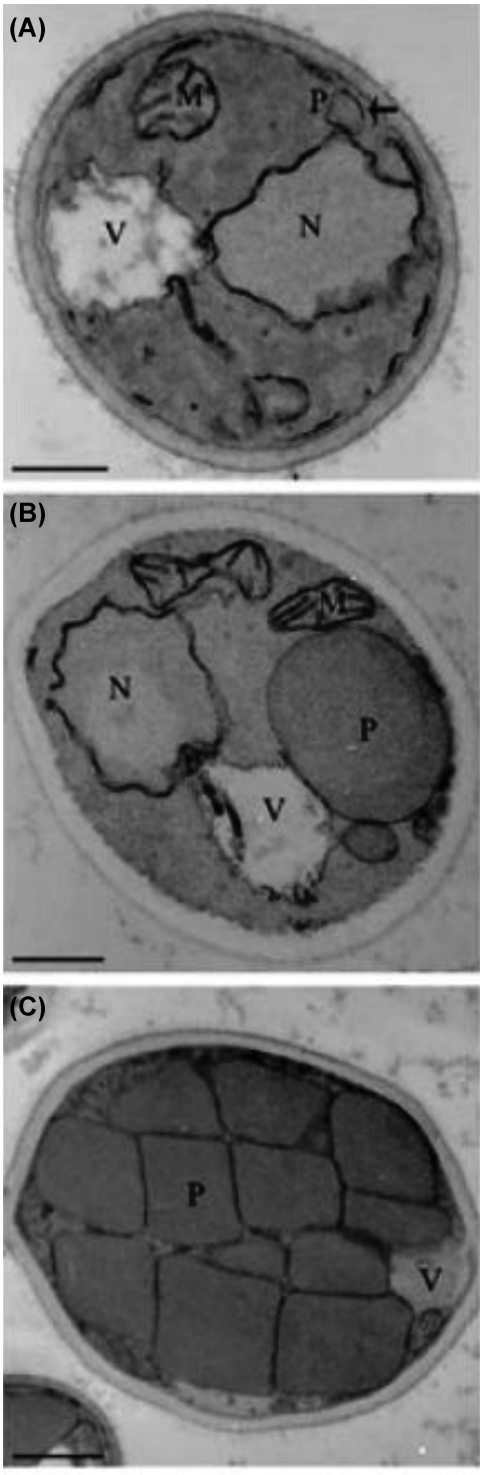

Figure 1. Peroxisomes in the cells of the methylotrophic yeast *Hansenula polymorpha* (Nagotu, Veenhuis and van der Klei 2010). (**A**) A cell grown in glucose medium (a small peroxisome is present). (**B**) A cell after shifting from glucose medium to methanol (the peroxisome has grown to a large size). (**C**) Cells after prolonged cultivation in methanol medium (massive proliferation of peroxisomes occurs).

some yeast species show dual localization (in the cytosol and peroxisomes) of the enzymes of the oxidative part of the pentose phosphate pathway, malate dehydrogenase and glycolysis (Freitag, Ast and Bölker 2012; Kabran *et al*. 2012; Strijbis *et al*. 2012). Such dual localization occurs due to alternative splicing of yeast introns, ribosomal read-through of stop codons or low-efficiency peroxisome targeting signals. Defects in peroxisome biogenesis lead to defects in utilization of the corresponding carbon sources, suggesting the importance of peroxisomal localization of the above-mentioned enzymes which previously were thought to be localized only in the cytosol (Idnurm *et al*. 2007; Stehlik *et al*. 2014). Most yeast catalases are localized in peroxisomes, and many species contain only peroxisomal catalases, although some others, e.g. *S. cerevisiae*, also possess cytosolic catalase (Jamieson 1998). In addition to catalase, yeast peroxisomes also contain another enzyme involved in detoxification of hydrogen peroxide, glutathione peroxidase, also known as Pmp20 protein (Horiguchi *et al*. 2001; van der Klei *et al*. 2006; Ohdate and Inoue 2012). Yeast peroxisomes also contain glutathione transferases, involved in reduction of disulfide bridges of oxidized proteins (Barreto *et al*. 2006). Enzymes involved in catabolism of unusual carbon and nitrogen sources (methanol, *n*-alkanes, purines, D-amino acids, methylamine, ethylamine, pipecolynic acid, sarcosine, glycolate, spermidine, etc.), as well as in lysine biosynthesis are also found in yeast peroxisomes (Breitling *et al*. 2002; Aksam *et al*. 2009; Sibirny 2012). Substrates of catabolic enzymes localized in peroxisomes often act as peroxisome proliferators by inducing peroxisome fission and increasing in size. Peroxisomes also contain two phosphatases, Npy1 and Pcd1, involved in metabolism of derivatives of the coenzymes NAD and CoA (Cartwright *et al*. 2000; Abdelraheim *et al*. 2001). In certain cases, peroxisomes can occupy a substantial part or even most of the cell volume, as can be observed in the methylotrophic yeast *H. polymorpha* during growth on methanol (Fig. 1) (Veenhuis, Van Dijken and Harder 1983; van der Klei *et al*. 2006; Nagotu, Veenhuis and van der Klei 2010).

Absence of peroxisomes leads to mislocalization of matrix peroxisomal enzymes in the cytosol. This has no impact on yeast growth on methylated amines or ethanol which are normally metabolized in peroxisomes (e.g. in *S. cerevisiae*; see Kunze *et al*. 2006). However, such mutants (including those of *S. cerevisiae*) are unable to grow on fatty acids or methanol. The reasons for this are not clear. Possibly, the cells are poisoned by H_2O_2 accumulated in the cytosol of peroxisome-deficient mutants (van der Klei, Harder and Veenhuis 1991). It is known that only some enzymes of the initial steps of methanol metabolism are localized in peroxisomes: alcohol oxidase, catalase and dihydroxyacetone synthase, whereas the others (formaldehyde dehydrogenase, S-formyl glutathione hydrolase, formate dehydrogenase, dihydroxyacetone kinase and other enzymes of the xylulose monophosphate pathway of formaldehyde assimilation) are located in the cytosol. It is interesting to note that dihydroxyacetone kinase of *P. pastoris* possesses peroxisome targeting signal 1 (PTS1; see below) despite the protein being localized in the cytosol (Lüers *et al*. 1998). It is thought that dihydroxyacetone kinase is folded into a conformation that makes its C-terminus inaccessible to the PTS1 receptor. Recently, it was suggested that *P. pastoris* contains all enzymes of the xylulose monophosphate cycle located in peroxisomes, so this species possesses isozymes of the non-oxidative part of the pentose phosphate pathway located in both the cytosol and peroxisomes (Rußmayer *et al*. 2015). It was also shown, contrary to previous findings (Lüers *et al*. 1998), that dihydroxyacetone kinase in *P. pastoris* is localized in both the cytosol and peroxisomes (Rußmayer *et al*. 2015). It was, however, definitely shown that elimination of the PTS1 signal in dihydroxyacetone kinase protein does not lead to any defects in methanol utilization, suggesting that this enzyme is indeed functionally active when located in the cytosol (Lüers *et al*. 1998). Also, it has to be pointed out that the functional role of peroxisomal enzymes of the xylulose monophosphate pathway remains to be elucidated. In *H. polymorpha*, mutants with knock out of peroxisomal transaldolase (*tal2*Δ) normally grow on methanol, in contrast to the mutants defective in cytosolic transaldolase (*tal1*Δ), which are totally defective in growth on this alcohol. Thus, the peroxisomal transaldolase at least is redundant for methanol utilization, whereas the cytosolic isoform is essential for this process (Kurylenko *et al*. 2016). In other words, proof of peroxisomal localization of the enzymes of the pentose phosphate pathway does not mean that they are involved in methanol metabolism *in vivo*.

PEROXINS AND PEROXISOME BIOGENESIS

Yeasts appear to be a convenient model to study the mechanisms of peroxisome biogenesis because cell transfer from a glucose-containing medium into a medium containing a peroxisome proliferator (oleate and/or methanol are mostly used) induces synthesis of peroxisomal enzymes and the growth and division of peroxisomes. Peroxisomes may occupy up to 80% of the cell volume in cells growing in the presence of methanol under certain conditions (Veenhuis, Van Dijken and Harder 1983; Nagotu, Veenhuis and van der Klei 2010). Peroxisomal biogenesis relies on the endoplasmic reticulum (ER) which provides lipids and peroxisomal membrane proteins (PMPs), and on the cytosol which supplies the matrix proteins. Currently, 34 genes whose products are specifically involved in peroxisome biogenesis have been identified (Supplementary Table S1). These are so-called *PEX* genes, whereas their products are known as Pex proteins or peroxins. Most of them have been identified on *S. cerevisiae*, although some peroxins are absent in this organism. Soluble peroxisomal matrix proteins (e.g. enzymes of fatty acid β-oxidation, alcohol oxidase, catalase, etc.) are not called peroxins. The majority of known *PEX* genes were identified by using yeast mutants defective in peroxisome biogenesis (Erdmann *et al*. 1989; Liu *et al*. 1992; Elgersma *et al*. 1993; Johnson *et al*. 1999), and recently further peroxins were identified by organelle proteomics (Pex11 and Pex13) or transcriptome analysis (Pex25) (Kohlwein, Veenhuis and van der Klei 2013). The mechanisms of action of peroxins were determined via phenotypic analysis of the mutants, the use of the yeast two-hybrid system and the development of methods for biochemical analyses of the proteins. The majority of peroxins are involved in the import of peroxisomal matrix proteins (Heiland and Erdmann 2005; Ma and Subramani 2009; Yuan, Veenhuis and van der Klei 2016). Major groups of peroxins include receptors Pex5 and Pex7 for peroxisomal matrix proteins containing PTS1 and PTS2 targeting sequences, respectively; peroxisome-associated docking proteins (Pex13) for Pex5; and another docking protein, Pex14, which binds to both Pex5 and Pex7 receptors. Three known as RING peroxins (Pex2, Pex10 and Pex12) are integral membrane proteins, possess zinc fingers and act as E3 ligases during receptor recyclization. Two proteins, Pex1 and Pex6, are ATPases of the AAA family. Pex4 belongs to the ubiquitin-conjugating enzymes; Pex11 is involved in peroxisome proliferation; Pex8 matrix protein contains both targeting sequences (PTS1 and PTS2) and forms a multiprotein complex involved

in peroxisomal protein import; two proteins, Pex16 and Pex17, are associated with the outer membrane surface and are involved in peroxisomal trafficking of PMPs via the ER most probably involved in the import of several matrix proteins; finally, several peroxins act as membrane proteins and are possibly involved in protein translocation across the membrane. Several peroxins are species specific. For instance, Pex16 and Pex33 are absent from methylotrophic yeasts and are only found in other ascomycetes (*S. cerevisiae*, *Y. lipolytica* and *Neurospora crassa*). Peroxin Pex26 is present only in mammals (Ma and Subramani 2009; Sibirny 2012; Aranovich *et al*. 2014; Kim and Hettema 2015).

All peroxisomal matrix proteins are translated on free polyribosomes in the cytosol and then post-translationally imported into pre-existing peroxisomes (Lazarow and Fujiki 1985). The term 'peroxisome biogenesis' implies mostly the processes by which the peroxisomal matrix and membrane proteins are assembled into the organelle, as well as those involved in the control of peroxisome size, volume and number (Ma and Subramani 2009). There are two types of PTS for peroxisomal soluble proteins, PTS1 and PTS2. PTS1, which is located at the C-terminus of proteins, is used by the majority of peroxisomal matrix proteins. Apart from some variants, the evolutionarily conserved sequence shared by PST1 is (S/A/C)-(K/R/H)-(L/M) (Gould *et al*. 1989; Lametschwandtner *et al*. 1998). A small group of matrix proteins are targeted to the peroxisome by PTS2 which is a conserved nonapeptide with the consensus sequence (R/K)-(L/V/I)-X5-(H/Q)-(L/A) (where X means any amino acid) and is located near the N-terminus (Rachubinski and Subramani 1995). Even more rarely proteins possess both PTS1 and PTS2 simultaneously, e.g. Pex8 of *P. pastoris* (Zhang, Léon and Subramani 2006). The PTSs for matrix proteins are recognized by specific cytosolic receptors which escort the cargos to the peroxisome membrane (Léon, Goodman and Subramani 2006a). Regions of the Pex5 receptor responsible for binding PTS1-containing cargos have been identified (Hagen *et al*. 2015). Subsequently, the matrix proteins and their receptors enter the peroxisome matrix (Nair, Purdue and Lazarow 2004; Leon *et al*. 2006b) where cargos are released, and the cargo-free receptors are first exported to the peroxisome membrane via a retro-translocation step, and then the PTS receptors are recycled (Ma and Subramani 2009). The sequence of events during matrix protein import is as follows: (i) cargo recognition and binding; (ii) docking of the receptor–cargo complex at the peroxisome membrane; (iii) cargo translocation and release; (iv) translocation of free receptors to the peroxisomal membrane; and (v) recycling of free receptors from peroxisome membranes to the cytosol (Ma and Subramani 2009). PTS1 of delivered cargo protein is recognized by the specific receptor cytosolic protein Pex5 which interacts with the cargo via tetratricopeptide repeats located at the C-terminus of Pex5 (Stanley *et al*. 2006). Cargo proteins which use PTS2 employ the protein Pex7 as receptor (Marzioch *et al*. 1994). Some PTS2 proteins interact with the Pex20 co-receptor (Otzen *et al*. 2005). Finally, there are data on matrix proteins that cross the peroxisomal membrane but do not contain PTS1 or PTS2 signals (Thoms *et al*. 2008, Thoms 2015). The PTS receptor–cargo complex interacts with a peroxisome membrane-associated docking complex comprised of the conserved proteins Pex13, Pex14 and, sometimes, Pex17 (Agne *et al*. 2003). It was believed that components of the docking and RING subcomplexes, as well as the proteins that bridge these subcomplexes (Pex3 and Pex8), formed an importomer, a term assumed to be synonymous with the translocon (Agne *et al*. 2003; Rayapuram and Subramani 2006). In the case of translocation of Pex8, peroxisomal membrane protein Pex14 could be the only protein required for cargo translocation (Ma and Subramani 2009). The mechanisms of cargo release remain unknown. Export of cargo-free receptors from inside peroxisomes apparently involves RING subcomplex proteins including Pex2, Pex10 and Pex12 (Agne *et al*. 2003; Leon *et al*. 2006b). The sequence of events during matrix protein import (Fig. 2) is as follows: the

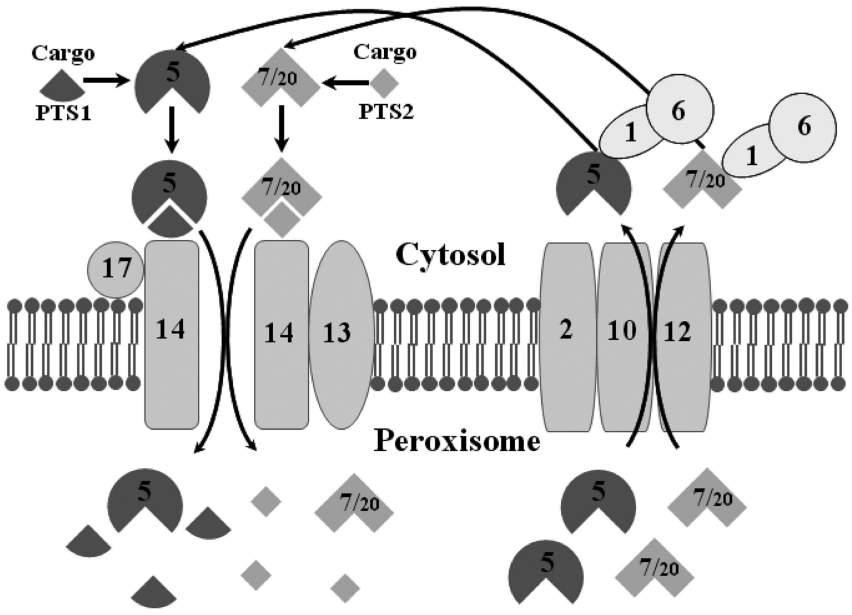

Figure 2. The scheme of the import of peroxisome matrix proteins. A cargo protein to be imported binds to a soluble receptor (Pex5 for PTS1-containing proteins or Pex7 and Pex20 for PTS2-containing proteins). The receptor–cargo protein complex binds to the so-called docking complex on the peroxisomal membrane consisting of proteins Pex13, Pex14 and Pex17, and the receptor–cargo protein complex is transferred into the peroxisomal matrix. The receptor–cargo protein complex dissociates in the peroxisomal matrix to release the cargo protein, whereas the receptor is exported onto the peroxisomal membrane with involvement of the proteins Pex2, Pex10 and Pex12. The receptors could be utilized in the next import round. The corresponding peroxins are indicated by their ordinal numbers.

peroxins Pex1 and Pex6 form heterohexamers and enable matrix protein translocation through the central channel of the Pex1/Pex6 oligomer by ATP hydrolysis (Ciniawsky *et al*. 2015; Gardner *et al*. 2015; Knoops *et al*. 2015). The dynein light chain protein Dyn2 is also implicated in matrix peroxisome targeting and biogenesis (Chang *et al*. 2013). Some peroxisomal matrix proteins do not possess PTSs and are transported due to binding to a protein with a PTS (Kumar *et al*. 2016).

Targeting of membrane peroxisomal proteins is tightly connected to the question of the origin of peroxisomes. It is interesting to note that mutants *pex3*, *pex16* and *pex19* that completely lack peroxisomal membrane structures produce normal peroxisomes upon rescue with wild alleles of the mutated *PEX* genes (though *pex3* mutants of *H. polymorpha* contain pre-peroxisomal vesicles) (Höhfeld, Veenhuis and Kunau 1991; Hoepfner *et al*. 2005; Knoops *et al*. 2014). It was hypothesized that newly formed peroxisomes originate from the ER (Tam *et al*. 2005). The details of membrane peroxin targeting remain unclear. It should be noted that there are two classes of membrane peroxins, those with tail anchor sequences (such as Pex15) and the predominant ones with one or several transmembrane domains (such as Pex2). Available data suggest that at least two routes exist by which PMPs can reach peroxisomes: one direct route and one via the ER (Kim and Hettema 2015). Most membrane peroxins are imported post-translationally from the cytosol. Many of these proteins possess a membrane PTS (mPTS) that consists of a cluster of positively charged residues or a mixture of positively charged and hydrophobic residues flanked by one or two transmembrane segments that are recognized and bound by Pex19 (Baerends *et al*. 2000; Wang, Unruh and Goodman 2001). One of the models for direct post-translational targeting of peroxisomal membrane proteins to peroxisomes proposes that Pex19 acts as a soluble recycling receptor/chaperone that picks up newly synthesized proteins in the cytosol and subsequently docks on Pex3 in the peroxisomal membrane (Muntau *et al*. 2003). The pathway of membrane peroxins through the ER was first described in *Y. lipolytica* (Titorenko, Ogrydziak and Rachubinski 1997). It is assumed that Pex3, Pex16 and Pex19 are involved in the early steps of the ER–peroxisome pathway and are the earliest membrane peroxins that are associated with the ER (Sacksteder *et al*. 2000). In *Y. lipolytica*, the newly synthesized membrane peroxin Pex16 is incorporated into the ER and provides a scaffold for binding of other peroxins (such as Pex3 and Pmp34) from the cytoplasm (Titorenko and Rachubinsky 1998). The process is somewhat different in yeasts lacking a *PEX16* homolog, e.g. in *S. cerevisiae*. For instance, Pex3 is initially targeted into the ER and then segregated into a pre-peroxisomal entity together with the Pex19 membrane peroxin, which acts as a receptor and, by interacting with Pex3, ensures the import of other membrane peroxins into the peroxisome (Platta and Erdmann 2007). Recently, the existence of two distinct pre-peroxisomal vesicles that arise from the ER has been found. Each type of vesicle carries part of the peroxisomal translocon complex. Their fusion leads to assembly of the full peroxisomal translocon and subsequent uptake of enzymes from the cytosol (van der Zand *et al*. 2012). The exact functions of the Pex1–Pex6 peroxin complex are not known. Possibly they are involved in the fusion of the above-mentioned pre-peroxisomal vesicles (van der Zand *et al*. 2012) or in recycling of the Pex5 receptor (Erdmann 2016).

It is assumed that lipids of the ER and certain membrane proteins are delivered into peroxisomes via a vesicular transport pathway, although current data suggest a direct transfer of phospholipids from the ER into peroxisomes without vesicular transport (Raychaudhuri and Prinz 2008).

Biogenesis of yeast peroxisomes depends not only on peroxins but also on specific transcription factors identified in methylotrophic yeasts *H. polymorpha* (Leao-Helder *et al*. 2003), *P. pastoris* (Lin-Ceregino *et al*. 2006) and *Candida boidinii* (Sasano *et al*. 2008). Methanol weakly induced peroxin *PEX* genes (up to 5-fold) in *H. polymorpha*, though it strongly induced genes of peroxisomal matrix proteins such as enzymes of fatty acid β-oxidation and the glyoxylic acid cycle (van Zutphen *et al*. 2010). Methanol most effectively induced expression of the *MPP1* gene coding for a transcription activator of peroxisome biogenesis (394-fold). Mpp1 protein belongs to the family of transcription regulators containing so-called zinc fingers [$Zn(II)_2Cys_6$]. Mutant *mpp1* was unable to utilize methanol as the sole carbon and energy source and was characterized by a very low level of peroxisomal proteins including those of methanol catabolism, and, one of them, dihydroxyacetone synthase, was totally absent. Methanol did not induce peroxisome proliferation in *mpp1* cells which contained only one peroxisome. Moreover this single peroxisome did not undergo autophagic degradation after a shift to glucose medium (Leao-Helder *et al*. 2003). The mechanism of transcription activation by Mpp1 protein remains unknown. The *MPP1* gene of *H. polymorpha* resembles transcription factor genes of other methylotrophic yeasts: *MXR1* of *P. pastoris* (Lin-Ceregino *et al*. 2006) and *TRM1* and *TRM2* of *C. boidinii* (Sasano *et al*. 2008; 2010), which appear to be essential for peroxisome biogenesis. Repression of peroxisome biogenesis by glucose and glycerol in *P. pastoris* is controlled by the specific transcriptional repressor Nrg1 (Wang *et al*. 2016).

METABOLITE TARGETING TO PEROXISOMES

Some preliminary information is available on mechanisms of metabolite transport through the peroxisomal membrane. In contrast to the inner mitochondrial membrane, which contains nearly 50 membrane transport proteins, the peroxisomal membrane contains no more than 10 proteins and only a few of them fulfill transport functions (Antonenkov and Hiltunen 2012). In contrast to, for example, lysosomal enzymes, the enzymes in peroxisomes do not show latency, i.e. they do not increase their activity after disruption of the organelle membrane by physical treatment or detergents. This was later confirmed by detection of the activity of peroxisomal enzymes (urate oxidase, L-α-hydroxyacid oxidase and D-amino acid oxidase) in digitonin-permeabilized rat hepatocytes (Verleur and Wanders 1993). The results were interpreted as an indication that the peroxisomal membrane is open to solutes and contains pore-forming proteins. However, several lines of evidence indicate that the peroxisomal membrane is impermeable to solutes, and the results of some *in vitro* experiments may represent artifacts caused by disruption of peroxisomes during preparation. Furthermore, a peroxisomal membrane channel protein (pore) has been described; it is formed by Pxmp2 protein and is responsible for penetration of low molecular weight metabolites, e.g. glyoxylic acid cycle metabolites (Antonenkov *et al*. 2009; Grunau *et al*. 2009; Antonenkov and Hiltunen 2012). It is interesting that the peroxin Pex11 known to be involved in peroxisome proliferation (Thoms and Erdmann 2005) also forms a pore in the peroxisomal membrane, thus acting as a non-specific transient receptor potential cation-selective channel (TRPM) (Mindthoff *et al*. 2016). It was found that the membrane of yeast peroxisomes is impermeable to NAD/H and acetyl-CoA under *in vivo* conditions (van Roermund *et al*. 1995; Kal *et al*. 1999). Substrates for β-oxidation enter peroxisomes via ATP-binding cassette (ABC) transporters of subfamily D and are activated by specific acyl-CoA

synthetases for further metabolism (Baker *et al*. 2015). Finally, an ATP transporter, which belongs to the superfamily of mitochondrial inner membrane solute carriers, was detected in the membrane of peroxisomes from different sources (Palmieri *et al*. 2001; Arai *et al*. 2008).

PEROXISOME DIVISION AND INHERITANCE

We discuss here current knowledge on peroxisome division, movement to the bud and retention in the mother cell. Peroxisomes divide during the cell cycle for maintenance (constitutive division); in addition, they divide during peroxisome proliferation induced by fatty acids, methanol, purines, D-amino acids or other peroxisome proliferators (regulated division). Though normally peroxisomes are formed by fission of previous peroxisomes, in the case of introduction of *PEX3* or *PEX19* genes to the corresponding knock out mutants, peroxisomes are formed *de novo* from membrane vesicles of ER origin (Veenhuis and van der Klei 2014). Peroxisome fission is controlled by Pex11 which is responsible for organelle elongation. Knock out of *PEX11* led to a small number of enlarged peroxisomes, whereas its overexpression resulted in numerous small peroxisomes in the cell (Opaliński *et al*. 2011). Regulation of Pex11 involvement in peroxisome fission could be achieved by protein phosphorylation (Knoblach and Rachubinski 2010). Peroxisomes are formed by fission of existing peroxisomes due to activities of the dynamin-related proteins with GTPase activities, Dnm1 and Vps1. Cells of the double *dnm1 vps1* mutant contain only one large peroxisome, and its fission is totally blocked (Hoepfner *et al*. 2001; Motley and Hettema 2007; Nagotu *et al*. 2008). Dnm1 is essential for peroxisome fission under induction of peroxisome proliferation, whereas Vps1 functions under glucose-repressing conditions (Hoepfner *et al*. 2001; Kuravi *et al*. 2006). The role of the tail-anchored protein Fis1 in Dnm1 recruitment to peroxisomes was established (Motley and Hettema 2007; Motley, Ward and Hettema 2008). The role of peroxins other than Pex11 in peroxisome fission was found, namely of peroxins of Pex11 family members (Pex25, Pex27 and Pex34) (Tower *et al*. 2011) and of Pex23 and its family members (Pex24, Pex28, Pex29, Pex30, Pex31 and Pex32) (Kiel, Veenhuis and van der Klei 2006).

During yeast cell budding, organelles, including peroxisomes, are duplicated and distributed between the mother and daughter cells, which is powered by the cytoskeleton. The actin-based movement of organelles is catalyzed by the class V myosins, mainly Myo2 (Knoblach and Rachubinski 2015). These molecular motors contain N-terminal domains that are required for binding to F-actin and generating force through the hydrolysis of ATP, while their C-termini form globular tails that attach to cargos by binding to organelle-specific adaptor molecules (Sellers and Veigel 2006). Myosin Myo2 is responsible for the movement of peroxisomes as well as secretory vesicles, Golgi, mitochondria and vacuoles (Fagarasanu *et al*. 2010). Lipid droplets also segregate to buds in a Myo2-dependent manner (Knoblach and Rachubinski 2015). Peroxisomes attach to Myo2 through inheritance of peroxisome protein 2 (Inp2), a peroxisomal integral membrane protein. Peroxisomes are transferred from the mother cell to buds in a highly ordered vectorial process (Fagarasanu *et al*. 2006). Moreover, Myo2 moves different organelles at distinct times in the cell cycle; for example, movement of peroxisomes always precedes movement of mitochondria (Knoblach *et al*. 2013). Mutagenesis of conserved surface amino acids in the Myo2 tail identified sequences required for its binding specifically to vacuoles, mitochondria, secretory vesicles and peroxisomes (Pashkova *et al*. 2006; Altmann *et al*. 2008; Fagarasanu *et al*. 2009). Mutants in which Myo2 can no longer attach to the peroxisomal surface do not exhibit bud-directed polarization of peroxisomes (Motley and Hettema 2007), and peroxisomes remain associated with the same cell over many generations because they are not released from their cortical tethers (Knoblach *et al*. 2013). These peroxisomes then also accumulate Inp2 and cannot be transported to the bud (Fagarasanu *et al*. 2009; Knoblach *et al*. 2013). Inp2 is the adaptor protein of Myo2 that forms a transport complex with peroxisomes. The level of Inp2 changes during the cell cycle; it reaches maximal concentration during peroxisome transfer to the bud and is degraded after their delivery (Fagarasanu *et al*. 2006; Peng and Weisman 2008). Inp2 contains several recognition sites for the cyclin-dependent kinase Cdk1 and is phosphorylated during the cell cycle (Peng and Weisman 2008; Fagarasanu *et al*. 2009). Peroxisome inheritance is uncoupled from the cell cycle in the Myo2 mutants defective in Inp2 binding; the levels of Inp2 are increased, implying that the amount of Inp2 is not controlled by the cell cycle but rather by the distribution of peroxisomes in the cell. Thus, peroxisome delivery between mother and daughter cells is precisely regulated.

It is important for at least a part of organelles, including peroxisomes, to remain in the mother cell during division. Peroxisome retention depends on their anchoring at the cell periphery (Fagarasanu *et al*. 2005). Organelles, including peroxisomes, are retained in the cell by the formation of membrane contact sites, or tethers, with other organelles. Organelle tethers assemble at discrete foci where membranes from two organelles come into close contact but do not fuse. Tethering proteins are either integral to the membrane of one of the compartments or can be classified as membrane-associated proteins that link the integral membrane proteins to each other. The structure of the tether that connects peroxisomes has recently been resolved. It consists of two proteins, Inp1 and the peroxin Pex3 (Knoblach *et al*. 2013). Cells lacking Inp1 contain only mobile peroxisomes that are eventually driven to the bud by the Inp2–Myo2 transport complex, whereas cells overproducing Inp1 maintain all of their peroxisomes in fixed cortical positions in the mother cell and do not transfer any peroxisomes to the daughter cell (Fagarasanu *et al*. 2005). Inp1 is required for attaching peroxisomes to Pex3, which is essential for peroxisome biogenesis and is integral to both the ER and the peroxisomal membranes (Hoepfner *et al*. 2005; Munck *et al*. 2009; Thoms *et al*. 2012). Inp1 contains at least two binding sites for Pex3 and acts as a molecular hinge by bridging ER-bound Pex3 and peroxisomal Pex3 into an ER–peroxisome tethering complex (Knoblach *et al*. 2013). Mutants defective in peroxisome division contain a single giant peroxisome (Hoepfner *et al*. 2001; Kuravi *et al*. 2006). Inp1 and Inp2 are present on this enlarged peroxisome but are polarized to opposite ends. Inp2 is enriched on the part of the peroxisome that protrudes into the bud, whereas Inp1 is found on the part that is retained in the mother cell (Knoblach *et al*. 2013). Cells lacking Inp1 have fewer and larger peroxisomes than wild-type cells, and cells overexpressing Inp1 contain numerous small peroxisomes. Inp1 physically interacts with peroxins Pex25, Pex30 and Vps1, proteins that modulate the elongation and scission steps of peroxisome division (Fagarasanu *et al*. 2005; Schrader, Bonekamp and Islinger 2012).

Substantial information is available on the interaction of the ER and peroxisomes, including PMP targeting and peroxisome origin from the ER (David *et al*. 2013; Dimitrov, Lam and Schekman 2013). Interaction of the peroxisome with vacuoles has been well investigated; it occurs during autophagic peroxisome

degradation. However, there is a little knowledge about the interaction or cross-talk between peroxisomes and mitochondria. Recently it has been found that peroxisomes establish physical contacts with mitochondria as they are localized to specific mitochondrial subdomains such as mitochondria–ER junctions and sites of acetyl-CoA synthesis (Cohen *et al*. 2014). It was recently reported that the membrane peroxin Pex11 physically interacts with mitochondrial Mdm34 and that such an interaction is important for contacts between peroxisomes and mitochondria through the ERMES complex (Mattiazzi Ušaj *et al*. 2015). Apposed membrane contact sites were also found between peroxisomes and lipid droplets and additionally with mitochondria (Pu *et al*. 2011; Schrader *et al*. 2015). These specific molecular interactions suggest involvements of peroxisomes and mitochondria in metabolism of lipid droplets in yeast.

PEROXISOME AUTOPHAGIC DEGRADATION (PEXOPHAGY)

Studying the mechanisms of autophagic degradation of cytosolic proteins and organelles, including peroxisome (pexophagy), is one of the hot topics of modern cell biology. There are numerous reviews on autophagy and pexophagy (Kiel 2010; Manjithaya *et al*. 2010; Sibirny 2011; Till *et al*. 2012; Suzuki 2013, 2014; Oku and Sakai 2016). Autophagy is a conserved mechanism of continuous degradation of proteins and organelles, including peroxisomes, though it is activated during nitrogen starvation. Apparently, it takes place during yeast propagation in any medium, contributing to cellular maintenance, housekeeping or as a turnover mechanism (Aksam *et al*. 2007; Suzuki 2013). However, usually pexophagy is a highly specific mechanism involved in degradation of excess peroxisomes, which, nevertheless, leaves at least one peroxisome non-degraded. As mentioned previously, yeasts, especially methylotrophic yeasts, are very convenient model systems to study pexophagy. A shift of glucose-grown cells into media with peroxisome proliferators, e.g. oleate and especially methanol, increases the cellular volume of peroxisomes up to 80%. When such cells from methanol- or oleate-containing medium are shifted to media with glucose or ethanol, massive degradation of peroxisomes occurs (Sakai *et al*. 2006; Sibirny 2014).

Most steps are shared between specific pexophagy and general (nonspecific) autophagy. The following proteins are involved (Manjithaya *et al*. 2010): (i) signaling proteins required for autophagy induction: protein kinase Tor1, protein kinase A, Sch 9, Tap42 and phosphatase type 2A; (ii) packaging proteins or organelles transported for degradation (Atg19, Atg11 and Atg8); (iii) formation of pre-autophagosomal structure (Atg1, Atg11, Atg13, Atg17, Atg29 and Atg31); (iv) vesicle nucleation (Atg6, Atg9 and phosphatidylinositol 3-kinase); (v) vesicle expansion and completion (Atg3-5, Atg6, Atg7, Atg8, Atg10, Atg12, Atg14 and Atg16); (vi) protein retrieval (Atg1, Atg2, Atg18, Atg23 and Atg27); (vii) homotypical fusion of isolation membrane (Tlg2); (viii) transport and heterotypical fusion of autophagosome and vacuoles (v- and t-SNAREs, Ccz1, Mon1 and HOPS complex); and (ix) intravacuolar vesicle degradation (Atg15, proteinase A and proteinase B).

The list of the known AuTophaGy-related (Atg) proteins is presented in Supplementary Table S2. Among the 42 currently known Atg proteins, only 17 are necessary for all types of autophagy, whereas the others are specific: either used in special pathways of selective autophagy or representing species-specific modifications. Specific pexophagy pathways utilize several specific proteins, which do not participate in non-specific autophagy. During pexophagy, the specific pre-autophagosomal structure (PAS) is formed, distinct from PASs produced during other types of selective autophagy. The pexophagy-specific PAS is organized by Atg11, Atg17 and Atg30 (Farré *et al*. 2008; Nazarko, Farré and Subramani 2009). Studyies of pexophagy in the methylotrophic yeast *P. pastoris* revealed two morphologically diverse pexophagy processes, called macropexophagy and micropexophagy (Manjithaya *et al*. 2010; Farré and Subramani 2004; Tuttle and Dunn 1995; Sakai *et al*. 2006; Sibirny 2011). During macropexophagy initiated by transferring cells from methanol medium to ethanol medium, individual peroxisomes are gathered in double membrane structures called pexophagosomes that merge with vacuoles, leading to degradation and repeated usage of the pexophagosomal content. During micropexophagy (occurring after transferring methylotrophically grown cells to glucose medium), peroxisome clusters are engulfed by vacuolar-sequestering membranes and a specific micropexophagy apparatus (MIPA) (Mukaiyama *et al*. 2004), which forms a cap above a cup-shaped vacuolar sequestering membrane surrounding a peroxisome (Farré *et al*. 2009; Manjithaya *et al*. 2010) (see Fig. 3). Heterotypical fusion between vacuolar-sequestering membranes and the MIPA transports peroxisomes inside the vacuole for degradation and repeated use of its components. The MIPA and pexophagosomes originate from the PAS. In *H. polymorpha*, nitrogen limitation leads to peroxisome degradation by a mechanism similar to micropexophagy. However, this process occurs due to a non-specific autophagic mechanism, by which cytosolic components are taken up by vacuoles concomitantly with peroxisomes, and was therefore named microautophagy of peroxisomes (Bellu *et al*. 2001b; van Zutphen, van der Klei and Kiel 2008).

There are several specific proteins involved only in pexophagy and not in the other types of autophagy: Atg24, Atg26, Atg28 and Atg30. In *P. pastoris*, Atg24 localizes to the pexophagosome–vacuole fusion complex during macropexophagy. This protein contains a PtdIns3P-binding module (Ano *et al*. 2005). A defect in PpAtg24 blocked pexophagy after pexophagosome formation and before fusion to the vacuole. Apparently PpAtg24 is involved in pexophagosome fusion with the vacuole and in micropexophagy. *ATG26* encodes an enzyme, sterol glucoside transferase (Oku *et al*. 2003; Stasyk *et al*. 2003), which is involved in pexophagy in *P. pastoris* but not in the alkane-utilizing yeast *Y. lipolytica*. It was found that in *P. pastoris*, *ATG26* was necessary for pexophagy of large peroxisomes, which accumulated in methanol medium (Nazarko *et al*. 2007a,b). It was also shown that the *P. pastoris* Atg26 was required for elongation of the PAS into the MIPA during micropexophagy (Yamashita *et al*. 2006). *Pichia pastoris ATG28* encodes a pexophagy-specific protein as its deficiency impairs both pexophagic mechanisms (macro- and micropexophagy) and only partially affects the general (non-specific bulk turnover) autophagy induced by nitrogen starvation (Stasyk *et al*. 2006; Nazarko, Farré and Subramani 2009).

Another pexophagy-specific protein is Atg30, which is involved in pexophagy and not in general autophagy due to interaction with peroxins Pex3 and Pex14. In *P. pastoris*, Atg30 interacts with Pex3 and Pex14 both localized on the peroxisomal membrane (Farré *et al*. 2008; Burnett *et al*. 2015). Effective peroxisome homeostasis probably requires their biogenesis and degradation to be co-ordinated. In *H. polymorpha* Pex14, the 64 N-terminal amino acid residues are necessary for pexophagy (Bellu *et al*. 2001a; van Zutphen, van der Klei and Kiel 2008). Apparently pexophagy is controlled by a receptor protein complex which consists of Pex3 and Pex14 peroxins, Atg30 receptor, Atg11 and

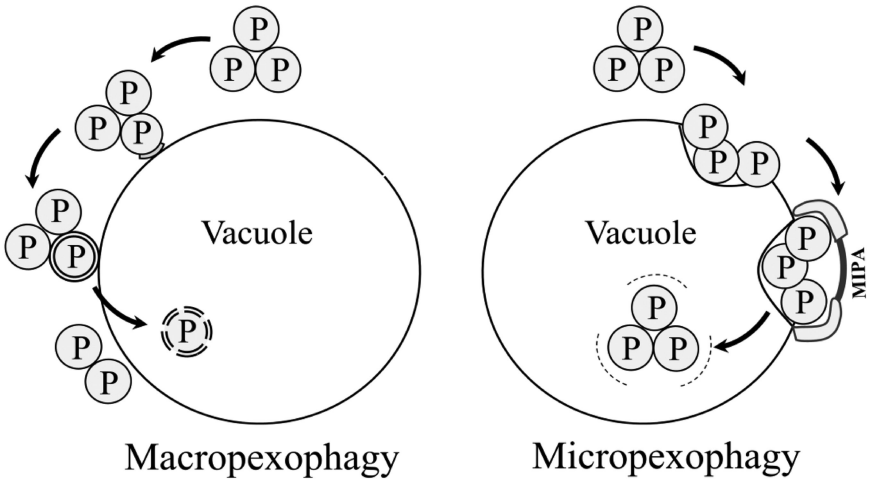

Figure 3. Schemes of macropexophagy (left) and micropexophagy (right). The formation of a pexophagosome around a peroxisome (P) is a distinguishing feature of macropexophagy. Micropexophagy includes a peroxisome (P) being engulfed by vacuolar membrane invagination with formation of a cup-shaped micropexophagy apparatus (MIPA).

Atg17 scaffolds, phagophore protein Atg8 and Atg37 involved in the assembly of this complex (Nazarko 2014). In *P. pastoris*, a gene designated *PDG1* (Peroxisome DeGradation) was identified, encoding the membrane protein whose mutations led to disturbances in peroxisome degradation (Dunn *et al.* 2005). In *H. polymorpha*, the transcriptional repressor Tup1 was shown to be essential for macropexophagy (Leão-Helder *et al.* 2004). Defects in orthologs of presumed repressors involved in glucose catabolite repression, *MIG1* and *MIG2*, also showed impairment in pexophagy (Stasyk *et al.* 2007). As mutants defective in *MIG1* and *MIG2* were not affected in glucose catabolite repression, one may assume that the functions of their products are different between baker's and methylotrophic yeasts. Over the last years, genes have been identified that are specifically involved in macro- and micropexophagy. The *ATG25* gene in *H. polymorpha* is specifically involved in macropexophagy. It codes for a coiled-coil protein that acts as a selectivity factor during macropexophagy (Monastyrska *et al.* 2005). This protein is located in the pexophagosomes and is transported there via the PAS. Atg25 is involved in the completion of sequestration of peroxisomes or in the fusion of pexophagosomes with the vacuolar membrane (Sakai *et al.* 2006). For the latter process, the SNARE Vam7 and the GTPase Ypt7 are also essential in *H. polymorpha* (Stevens *et al.* 2005).

The gene *PFK1* codes for phosphofructokinase 1 α-subunit, which is required for peroxisome engulfment by vacuoles after transferring *P. pastoris* cells from methanol to glucose medium (Yuan *et al.* 1997). The participation of phosphofructokinase 1 α-subunit in micropexophagy does not depend on its ability to phosphorylate fructose-6-phosphate since a catalytically inactive form of this enzyme allows for normal pexophagy. Moreover, the *VAC8* gene (VACuole related) was identified that specifically participates in micro- but not macropexophagy (Fry *et al.* 2006; Nazarko *et al.* 2007a). In the mutant cells, the vacuolar sequestering membrane is not formed during micropexophagy. Vac8 probably participates in early (formation of sequestering membrane) and late (membrane fusion after formation of the MIPA) micropexophagy stages. Mutations in the genes *PpGCN1*, *PpGCN2*, *PpGCN3* or *PpGCN4* involved in general amino acid control specifically inhibit micropexophagy after incorporation of the peroxisomes into the vacuole (Mukaiyama *et al.* 2002; Sakai *et al.* 2006), but the detailed functions of the Gcn proteins are not clear. The micropexophagy-specific protein Atg35, the first autophagy protein with nuclear localization, was identified in *P. pastoris* (Nazarko *et al.* 2011). Characterization of *P. pastoris atg35* mutants showed that macropexophagy was normal, whereas micropexophagy was impaired. It was found that Atg35 is necessary only for micropexophagy at the stage of MIPA formation (Nazarko *et al.* 2011). It is interesting that overexpression of *ATG35*, as well as deletion of this gene, both inhibit micropexophagy. Atg35 contains a putative nuclear localization signal. Atg35 localization on single dot-like structures of the nuclear membrane in glucose medium was found to be dependent on Atg17 and is significant for the micropexophagy process. Atg28 is known to interact with Atg17 (Nazarko *et al.* 2007a) and Atg35 (Nazarko *et al.* 2011).

We have limited knowledge on glucose and ethanol signaling involved in pexophagy initiation. In *P. pastoris*, *GSS1* coding for a glucose sensor was found to be important for pexophagy (Polupanov, Nazarko and Sibirny 2012; Polupanov and Sibirny 2014). Orthologs of GPCR genes *GPR1* and *GPA2* in *P. pastoris* are not involved in pexophagy, in contrast to the case in *S. cerevisiae* (Nazarko *et al.* 2008; Nazarko, Thevelein and Sibirny 2008). MAPK Slt2 protein is involved in glucose signaling during pexophagy in *P. pastoris* (Manjithaya *et al.* 2010).

PERSPECTIVES OF BIOTECHNOLOGICAL APPLICATIONS OF PEROXISOMES

Peroxisomes have been proposed to be used for expression of heterologous proteins. The advantage of storage of heterologous proteins in the peroxisomal matrix is the absence of protein-modifying enzymes in this cell compartment (e.g. mediating phosphorylation, glycosylation or proteolysis), which may give rise to undesired modifications upon production in the cytosol or during passage in the ER. Peroxisome could be an especially promising compartment for expression and storage of toxic proteins, as the peroxisomal membrane prevents their leakage out of the organelle. This is, however, only speculation, since reviews about the expression of heterologous proteins in *H. polymorpha* (Faber *et al.* 1996; van Dijk *et al.* 2000) and *P. pastoris* (Cereghino and Cregg 2000) do not give corresponding examples of the expression of heterologous industrial proteins in

peroxisomes. At least in one case, the presence of peroxisomes decreased the expression of a heterologous protein; shown for hepatitis B surface antigen in *H. polymorpha* (Krasovska, Stasyk and Sibirny 2013). There is also a possibility of locating several foreign enzymes to peroxisomes, thus creating a new metabolic pathway. This is a quite interesting and promising idea; however, transport of substrates of the pathway into peroxisome and exit of the product from the organelle could pose a problem. As was discussed above, many low molecular weight substances freely penetrate the peroxisomal membrane in both directions through the peroxisomal channel (pore) protein Pxmp2, whereas large molecular weight metabolites, such as coenzymes, penetrate the peroxisome via specific transport proteins (Antonenkov and Hiltunen 2012). To control the penetration of the required metabolite, a pore channel protein could be engineered and specific proteins for peroxisome transport could be modified for transport to peroxisomes; however, this possibility has not been explored yet. There is an interesting series of studies on expression of a heterologous pathway for penicillin biosynthesis from the mycelium fungus *Penicillium chrysogenum* in the methylotrophic yeast *H. polymorpha* (Gidijala *et al.* 2007, 2008, 2009). Synthesis of penicillin involves four genes; two of them are encoded cytosolic and the two others are peroxisomally localized enzymes. Expression of all four genes from *P. chrysogenum* in *H. polymorpha* resulted in yeast strains capable of penicillin synthesis. Expression of all four genes in the *pex3* mutant defective in peroxisome biogenesis resulted in a substantial drop in penicillin synthesis. In a similar manner, peroxisomes appeared to be important for penicillin synthesis by the native producer *P. chrysogenum* (Meijer *et al.* 2010). In general, synthesis of many fungal secondary metabolites occurs at least partially in peroxisomes (Stehlik *et al.* 2014). Yeasts rarely produce secondary metabolites, although they could be useful hosts for heterologous expression of the corresponding fungal genes, similarly to what was done for penicillin. Yeast peroxisomes appeared to be a suitable organelle for locating enzymes involved in synthesis of polyhydroxyalkanoates (Poirier, Erard and MacDonald-Comber Petétot 2002). It was suggested that peroxisomes could be interesting hosts for biochemical pathways involving CoA-linked intermediates (Stehlik *et al.* 2014).

As was pointed out above, peroxisomes are important for yeast catabolism of such unusual carbon and nitrogen sources as fatty acids, methanol, D-amino acids, purines, etc. Until recently, the role of peroxisomes in sugar catabolism in yeast was not known. Knock out of *DAS1*, coding for peroxisomal transketolase, known also as dihydroxyacetone synthase, and *TAL2*, coding for peroxisomal transaldolase, did not have any effects on growth on xylose, although it almost totally blocked xylose alcoholic fermentation, similarly to the effect of *pex3* and *pex6* mutations, with no effect on glucose fermentation. At the same time, overexpression of *DAS1* and *TAL2* genes, separately or together, strongly activated ethanol production from xylose. Simultaneous overexpression of *DAS1* and *TAL2* in the advanced ethanol producer from xylose (Kurylenko *et al.* 2014, 2016) led to further improvement of the main parameters of xylose alcoholic fermentation (yield and productivity) under elevated temperatures of 45°C, suggesting the important role of peroxisomes in maintaining a high efficiency of xylose alcoholic fermentation. It is not known at the moment whether peroxisomes are specifically involved in control of xylose growth and fermentation only in methylotrophic yeasts or also in other yeast organisms. It was also found out that deletion or disruption of the *H. polymorpha ATG13* gene encoding autophagy and pexophagy-initiating protein activates xylose (but not glucose) alcoholic fermentation (Kurylenko *et al.* 2016). It could be hypothesized that Atg13 protein is somehow specifically involved, in addition to its role in autophagy, in the control of xylose fermentation in methylotrophic yeasts. The mechanisms of this involvement in xylose metabolism and fermentation remain to be analyzed.

There are interesting data on the importance of yeast general non-specific autophagy in food biotechnology, e.g. during the second fermentation used for production of sparkling wines (Cebollero and Gonzalez 2007). Inhibition of autophagy by benzoic acid could be one of the reasons this compound prevents growth of food-spoiling yeasts (Abeliovich and Gonzalez 2009). To my knowledge, there are very few data on the role of the specific autophagic degradation of peroxisomes (pexophagy) in biotechnology, which includes only the recent finding on activation of xylose fermentation in *atg13*Δ mutants of *H. polymorpha* (Kurylenko *et al.* 2016). Still, an important role for pexophagy could be easy envisaged: if the metabolic process were to reside inside the peroxisome, it would be important to prevent or slow down the rate of autophagic degradation of this organelle. Therefore, manipulation of the pexophagy process (its retardation or total genetic block) appears to be a very promising approach for future yeast biotechnology.

GENERAL CONCLUSIONS

The data gathered in this review show that the many aspects of peroxisome biogenesis and protein targeting to this organelle are understood in great detail. This is also the case regarding pexophagy, i.e. the autophagic degradation of peroxisomes. The corresponding studies are among the top focus areas in modern cell biology. Nevertheless, our understanding of the mechanisms of peroxisome biogenesis, division, inheritance and pexophagy are still incomplete, and many important details remain to be elucidated in the near future. We do not know exactly how the size and number of peroxisomes is maintained, when peroxisomes originate from the ER and how cells decide to start peroxisome division. Another intriguing question is the mechanism providing maintenance of at least one peroxisome during massive pexophagy induced, for example, after a shift of methanol-grown cells to glucose medium in methylotrophic yeasts. Yeast peroxisomes could be promising compartments for expression of foreign biotechnologically important proteins or complete metabolic pathways for production of certain metabolites. Peroxisomes are important for catabolism of numerous carbon and nitrogen sources in yeasts and could be involved in biosynthesis of some primary and secondary metabolites. Recent data show the importance of this organelle for metabolism of the most abundant pentose and the second most abundant carbon source in nature after glucose, xylose. Modulation of activities of peroxisomal enzymes and peroxisome volume could be promising approaches for activation of xylose alcoholic fermentation, which is an important biotechnological issue.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSYR online.

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