



Peroxisomes

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Peroxisomes are among the simplest of the subcellular organelles that are characteristic of all eukaryotic cells. With ~60 known enzymes in the matrix and ~45 documented integral or peripheral membrane proteins, it is a reasonable guess that this organelle has only ~125 proteins, which makes it much less complex than other organelles. The peroxisome derives its name from the fact that many metabolic enzymes that generate hydrogen peroxide as a by-product are sequestered here because peroxides are toxic to cells. Within peroxisomes, hydrogen peroxide is degraded by the enzyme, catalase, to water and oxygen. Peroxisomes are surrounded by a single membrane and they range in diameter from 0.1 to 1 μm . They exist either in the form of a network of interconnected tubules (peroxisome reticulum), as in liver cells, or as individual microperoxisomes in other cells such as tissue culture fibroblasts.

Peroxisome-Like Organelles

Peroxisomes are related to specialized peroxisomes called glycosomes in parasites such as Trypanosomes, and to plant glyoxysomes, but are unrelated to hydroxysomes, mitochondria, and chloroplasts. Collectively, peroxisomes, glyoxysomes, and glycosomes are also referred to as microbodies.

Peroxisome Distribution and Origin

Peroxisomes exist in all eukaryotes from single- and multicellular microorganisms, to plants and animals. Unlike mitochondria, nuclei, and chloroplasts, peroxisomes have no DNA. Consequently all their proteins are encoded by nuclear genes. They are proposed to have originated from endosymbionts that subsequently lost their DNA, but the evidence for an endosymbiont origin is much weaker than it is for mitochondria and chloroplasts.

Regulation of Peroxisome Number, Volume, and Contents

Peroxisomes can be induced to proliferate in many organisms in response to metabolic needs. Examples

include the induction of proliferation of hepatic peroxisomes by fibrates drugs, phthalate plasticizers and xenobiotics, or the induction of peroxisomes in the methylotrophic yeast, *Pichia pastoris*, by methanol or oleate. The contents of the organelle are also responsive to the environment, as illustrated by the fact that peroxisomes of yeasts grown on oleate have induced levels of the fatty acid β -oxidation enzymes, whereas methylotrophic yeasts grown on methanol have elevated levels of alcohol oxidase and dihydroxyacetone synthase. Peroxisome volume can also be regulated by proteins such as Pex11p and Pex25p. Some mechanism must also exist for monitoring the need for peroxisomes and their function, because excess peroxisomes can be turned over by autophagic mechanisms involving the lysosome in mammals, or its yeast equivalent, the vacuole.

Functions of Peroxisomes

The principal function of peroxisomes is to house many metabolic pathways that are involved in various aspects of lipid metabolism. These include the following:

1. enzymes involved in the degradative oxidation (e.g., β -oxidation of very long chain fatty acids, 2-methyl-branched fatty acids, dicarboxylic acids, leukotrienes, bile acid intermediates and cholesterol side chains, and both α - and β -oxidation of 3-methyl-branched chain fatty acids);
2. the early steps in the synthesis of ether glycolipids or plasmalogens;
3. the formation of bile acids, dolichol, and cholesterol; and
4. the catabolism of purines, polyamines, and amino acids, and the detoxification of reactive oxygen species such as hydrogen peroxide, superoxide anions, and epoxides. In methylotrophic yeasts, peroxisomes are also involved in the metabolism of methanol and methyl amines.

Glycosomes contain the glycolytic enzymes, in addition to enzymes common to most peroxisomes, whereas plant glyoxysomes have some or all of the

glyoxylate pathway enzymes. Peroxisomes in the leaves of plants also participate in photorespiration.

Despite the fragility of the organelle during biochemical purification, the peroxisome membrane is impermeable to small molecules such as NAD(H), NADP(H), acetyl-CoA, and even protons *in vivo*. Consequently, it is not surprising that the peroxisomal membrane has a number of transporters for fatty acids, fatty-acyl-CoA esters, metabolites, and ATP.

Involvement in Human Disease

There are ~20 peroxisomal disorders, many of which are fatal. These diseases affect either a single metabolic enzyme, or the assembly of the organelle itself (Table I). Almost all of the genes involved in the human peroxisome biogenesis disorders (PBDs) are now known – they fall within the 25 *PEX* genes required for peroxisome biogenesis. Mouse models for human PBDs offer the promise of better insights into the symptoms of these diseases, their diagnoses, and eventually for therapeutic intervention.

Biogenesis of Peroxisomes

Because peroxisomes have no DNA, all their proteins must be imported from genes encoded in the nucleus.

Most of proteins that reside in the peroxisome matrix and membrane are synthesized in the cytosol and then imported posttranslationally to the organelle. About 25 *PEX* genes, encoding proteins named peroxins, are necessary for the biogenesis of the organelle. Most of these genes are found in multiple organisms and 13 are conserved in humans (Table II). The general principles of biogenesis appear to be common to organisms across the evolutionary spectrum, but there are indeed organism-specific variations. More recently, additional *PEX* genes (*PEX23–PEX32*) have been defined and many of these are involved in the control of peroxisome division and number, rather than in protein import.

PEROXISOMAL MATRIX PROTEIN IMPORT

Proteins destined for the peroxisome matrix have a few peroxisome targeting signals (PTSs). Most matrix polypeptides have a conserved, C-terminal, tripeptide PTS1 (-SKL in the one letter amino-acid code, or its conserved variants). Others have an N-terminal or internal sequence termed PTS2 [(R/K) (L/V/I)X₅(H/Q) (L/A)]. A few proteins, such *Saccharomyces cerevisiae* acyl-CoA oxidase, either have no canonical PTS1 sequence or have one that is dispensable, suggesting that they may possess other, as yet undefined, features that allow them to be targeted to the peroxisome lumen.

Matrix proteins synthesized in the cytosol are bound by cytosolic receptors – Pex5p in the case of PTS1, and

TABLE I

Human Peroxisomal Disorders Involving Metabolism and Biogenesis

Disease	Peroxisomal enzyme affected
<i>Peroxisomal metabolic disorders</i>	
Pseudoneonatal adrenoleukodystrophy	Acyl-CoA oxidase (Acox1)
Multifunctional protein 2 (MFP2) deficiency	MFP2 involved in β -oxidation of very long chain and 2-methylbranched fatty acids
Peroxisomal thiolase deficiency	3-ketoacyl-CoA-thiolase
X-linked adrenoleukodystrophy	ALDP (transporter)
Rhizomelic chondrodysplasia punctata Type 2	Dihydroxyacetonephosphate acyltransferase
Rhizomelic chondrodysplasia punctata Type 3	Alkyl-dihydroxyacetonephosphate synthase
Refsum's disease (classical)	Phytanoyl-CoA hydroxylase
Glutaric aciduria Type 3	Glutaryl-CoA oxidase
Hyperoxaluria Type I	Alanine:glyoxylate aminotransferase
Acatalasaemia	Catalase
Mevalonic aciduria	Mevalonate kinase
Di/trihydroxycholestanic acidaemia	Trihydroxycholestanoyl-CoA oxidase (Acox2)
Mulibrey nanism	TRIM37
Adult-onset sensory motor neuropathy	2-methylacyl-CoA racemase
<i>Disease</i>	
<i>Peroxisome biogenesis disorders</i>	
Zellweger syndrome	Peroxin affected
Neonatal adrenoleukodystrophy	Pex1, Pex2, Pex3, Pex5, Pex6, Pex10, Pex12, Pex13, Pex16, Pex19
Infantile Refsum's disease	Several peroxins (Pex1, Pex5, Pex6, Pex10, Pex12, Pex13)
Rhizomelic chondrodysplasia punctata Type I	Several peroxins (Pex1, Pex2, Pex5, Pex12)
	Pex7

TABLE II

Proteins Involved in Peroxisome Biogenesis

Pex1	A 100–50 kDa ATPase (AAA family) in yeasts and humans. Interacts with Pex6 and other peroxins. Defects in Pex1 are the most common cause of the PBDs (CG1).
Pex2	A ~40 kDa integral PMP with a carboxy-terminal, cytoplasmically exposed, zinc-binding RING domain. Has been identified in yeasts and humans, interacts with Pex3, Pex10 and Pex12 and is defective in CG10 of the PBDs.
Pex3	A ~40 kDa integral PMP in yeast and humans that binds Pex19 and is defective in CG12 of the PBDs. Needed for assembly/stability of the RING-domain subcomplex comprised of Pex2, Pex10, Pex12 in yeast.
Pex4	A 20–24 kDa peroxisome-associated ubiquitin-conjugating enzyme that interacts with Pex22. Has been identified in several yeast species, but not in any metazoan.
Pex5	A ~70 kDa, predominantly cytoplasmic/partly peroxisomal protein that is found in yeasts, plants and humans. Contains a PTS1-binding, tetratricopeptide-repeat (TPR) domain in its carboxy-terminal half, interacts with several peroxins (Pex7, Pex8, Pex10, Pex12, Pex13 and Pex14) and is defective in CG2 of the PBDs.
Pex6	A ~100 kDa (AAA family) ATPase found in yeasts, plants and humans. Interacts with Pex1 and is defective in CG4 of the PBDs.
Pex7	A ~40 kDa, WD40-repeat-containing protein that binds the PTS2. Defective in CG11 of the PBDs.
Pex8	A variably sized (60–80 kDa), peripheral, but intraperoxisomal, PMP that interacts with Pex5 and the docking subcomplex, found only in yeast. It is an intraperoxisomal organizer of the peroxisomal import machinery in <i>S. cerevisiae</i> .
Pex9	A 44 kDa integral PMP found only in the yeast <i>Yarrowia lipolytica</i> .
Pex10	A ~35 kDa integral PMP with a carboxy-terminal, cytoplasmically-exposed, zinc binding RING domain. Has been identified in yeasts and humans, interacts with Pex2, Pex3, Pex5 and Pex12, and is defective in CG7 of the PBDs.
Pex11	A ~25 kDa integral PMP required for normal peroxisome abundance. Many species contain several Pex11 genes.
Pex12	A ~40 kDa integral PMP with a carboxy-terminal, cytoplasmically-exposed, zinc-binding RING domain. Has been identified in yeasts and humans, interacts with Pex2, Pex3, Pex5 and Pex10, and is defective in CG3 of the PBDs.
Pex13	A ~44 kDa integral PMP with a carboxy-terminal, cytoplasmically-exposed SH3 domain. Identified in yeasts and humans, interacts with Pex5 and Pex14, and is defective in CG13 of the PBDs.
Pex14	A ~40 kDa PMP of yeasts, plants and humans that interacts with Pex5, Pex8, Pex13 and Pex17.
Pex15	A 44 kDa integral PMP identified only in <i>Saccharomyces cerevisiae</i> . Interacts with Pex6 in yeast.
Pex16	In humans, Pex16 is a 36 kDa integral PMP that binds Pex19 and is defective in CG9 of the PBDs.
Pex17	A ~25 kDa integral PMP that interacts with Pex14. Has been identified only in <i>S. cerevisiae</i> and <i>P. pastoris</i> .
Pex18	A 31 kDa soluble protein involved only in PTS2-protein import. It is highly similar to Pex21, and might act as a Pex7 chaperone. Identified only in <i>S. cerevisiae</i> .
Pex19	A 33 kDa farnesylated protein of yeasts and humans. Predominantly cytoplasmic/partly peroxisomal, binds all known integral PMPs and recognizes some, but not all, mPTSs. It is defective in CG14 of the PBDs.
Pex20	A 46 kDa soluble protein involved only in PTS2-protein import. Identified only in <i>Y. lipolytica</i> . Substitutes functionally for Pex18/Pex21 in <i>S. cerevisiae</i> .
Pex21	A 31 kDa soluble protein involved only in PTS2-protein import, is highly similar to Pex18 and might act as a Pex7 chaperone. Identified only in <i>S. cerevisiae</i> .
Pex22	A 20 kDa integral PMP of yeasts that interacts with Pex4 and anchors it on the peroxisomal membrane.
Pex23	A 46 kDa integral PMP. Identified only in <i>Y. lipolytica</i> .
Pex24	A 61 kDa integral PMP found in yeasts required for the proper localization of some, but not all, PMPs and matrix proteins.
Pex25	A 45 kDa PMP found in <i>S. cerevisiae</i> that regulates peroxisome size and maintenance.

CG, complementation group; SH3, Src-Homology 3.

Pex7p in the case of PTS2 (see Figure 1). These receptor-cargo complexes then move to the peroxisome membrane where they dock with protein subcomplexes that are in or on the membrane. Two such complexes are a docking subcomplex, comprised minimally of peroxins Pex8p, Pex13p, Pex14p, and Pex17p, and a really interesting new gene (RING)-protein subcomplex, consisting of three RING-proteins Pex2p, Pex10p, and Pex12p (and other yeast peroxins, such as Pex3p and Pex8p). The RING-proteins have a characteristic zinc-binding domain and are members of a protein family whose first member was called RING. There is evidence that the protein subcomplexes are dynamic, e.g., the docking and RING-protein subcomplexes can come together as a larger complex during import. The PTS receptors, Pex5p and Pex7p, are believed to shuttle from the cytosol to the peroxisome membrane or lumen

of the organelle, where they release their cargo, before they return back to the cytosol for another round of import. This is referred to as the extended shuttle model for matrix protein import. It is unclear at present whether the RING-protein subcomplex (also called the translocation complex in the literature) is involved directly in the translocation of proteins into peroxisomes, or in the shuttling of receptors (e.g., Pex5p) back to the cytosol. Many other peroxins and chaperones such as Dj1p, hsp70, and hsp40 are implicated in matrix protein import, but their precise roles are still under investigation.

The PTS2 pathway, which is dependent on the receptor, Pex7p, requires different additional proteins depending on the organism of its origin. In *S. cerevisiae*, the redundant proteins, Pex18p and Pex21p, fulfill this function, whereas in *Yarrowia lipolytica*, Pex20p is

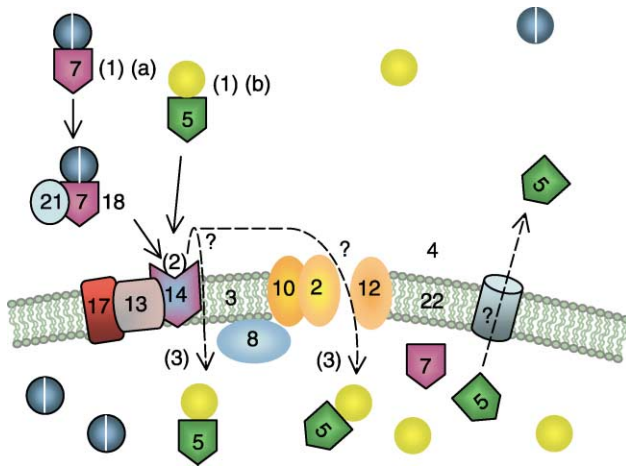


FIGURE 1 Model of peroxisomal matrix enzyme import. Numbers indicate the corresponding Pex protein. Three main steps are outlined: (1) Binding of PTS-containing proteins (yellow and blue circles depict PTS1- and multimeric PTS2-containing proteins, respectively) to the import receptors (Pex5p and Pex7p); (2) transport of receptor-cargo complexes to the peroxisome membrane and interactions with PMPs, such as Pex14p and, perhaps Pex13p, which are in a subcomplex with Pex17p and Pex8p; (3) receptor-cargo translocation through a proteinaceous pore formed either by the docking subcomplex (Pex14p, Pex13p, Pex17p, Pex8p) or the RING-peroxins subcomplex (Pex2p, Pex10p, Pex12p). PTS receptors may deliver cargo while inserted in the peroxisomal membrane or after entry into the lumen. Pex3p and Pex8p have been proposed to bridge proteins in the docking and RING-peroxins subcomplexes.

needed, and in mammals an alternatively spliced form of Pex5p (Pex5pL) is necessary for PTS2 import. However, the docking and RING-proteins are required in common for both PTS1 and PTS2 import pathways, leading to the current view that a common translocation machinery is involved for both these pathways.

Examples of organism-specific variations in the general scheme of biogenesis include the apparent lack of the entire PTS2 pathway (PTS2 proteins and Pex7p, the PTS2 receptor) in worms (*Caenorhabditis elegans*), and the dependence of the PTS2 import pathway on the PTS1 receptor, Pex5p, in mammals, but not in yeasts. However, even where such differences exist, the underlying molecular mechanism is similar. This is illustrated by the point that the proteins Pex18p and Pex21p from *S. cerevisiae*, Pex20p from *Y. lipolytica*, and Pex5pL in mammals all have a conserved motif that allows them to interact with Pex7p and/or PTS2 cargo to facilitate the PTS2 import pathway.

Unlike the transport of unfolded proteins into other organelles, such as the endoplasmic reticulum and mitochondria, folded and oligomeric proteins can be transported across the peroxisomal membrane. How such large multi-subunit complexes are transported across the membrane is unknown, because the translocon in the peroxisomal membrane has not been characterized.

IMPORT OF PEROXISOMAL MEMBRANE PROTEINS

These proteins have one or more sequences (mPTSs) that direct them to the peroxisomal membrane with the correct topology. Although a dozen or so mPTSs have been defined in several yeast and mammalian peroxisomal membrane proteins (PMPs), they have no simple consensus sequence. The PMP receptor(s), the mechanism of insertion of PMPs into the peroxisomal membrane, and the rules that govern their topology are not completely known, although several peroxins that play a role in PMP biogenesis are defined. Most mutants affecting the import of either peroxisomal matrix or membrane proteins have organelle remnants, in some but perhaps not all, organisms.

Division and Proliferation of Peroxisomes

The division of peroxisomes is compatible with two models. One of these proposes that peroxisomes arise by budding and fission from pre-existing peroxisomes, and may be the one that is used in normal, mitotically dividing cells. The other model is that peroxisomes arise either *de novo* or from some other reservoir of membranes such as the endoplasmic reticulum. Mature peroxisomes are then generated from this membrane reservoir via a variety of biogenesis intermediates. This model may be more applicable to proliferating peroxisomes and to the regeneration of peroxisomes in *pex* mutants complemented by the affected gene.

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GLOSSARY

autophagy Degradation of cytosol and organelles by protein turnover involving the yeast vacuole or the lysosome in mammals.
biogenesis The process of assembly.
microbodies Another name for peroxisomes and similar organelles (glyoxysomes, glycosomes).

organelle A subcellular, membrane-enclosed compartment performing specialized functions.

peroxisomal matrix Lumen of the peroxisome.

peroxisome A subcellular organelle involved in many lipid metabolic pathways.

vacuole or lysosome Organelle in which protein turnover and recycling occurs. The organelle is called the vacuole in yeast and the lysosome in mammalian cells.

FURTHER READING

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