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Review

The birth of yeast peroxisomes☆

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ABSTRACT

This contribution describes the phenotypic differences of yeast peroxisome-deficient mutants (*pex* mutants). In some cases different phenotypes were reported for yeast mutants deleted in the same *PEX* gene. These differences are most likely related to the marker proteins and methods used to detect peroxisomal remnants. This is especially evident for *pex3* and *pex19* mutants, where the localization of receptor docking proteins (Pex13, Pex14) resulted in the identification of peroxisomal membrane remnants, which do not contain other peroxisomal membrane proteins, such as the ring proteins Pex2, Pex10 and Pex12. These structures in *pex3* and *pex19* cells are the template for peroxisome formation upon introduction of the missing gene. Taken together, these data suggest that in all yeast *pex* mutants analyzed so far peroxisomes are not formed de novo but use membrane remnant structures as a template for peroxisome formation upon reintroduction of the missing gene. The relevance of this model for peroxisomal membrane protein and lipid sorting to peroxisomes is discussed. This article is part of a Special Issue entitled: Peroxisomes.

1. Introduction

Peroxisomes are morphologically simple organelles that measure up to 1 μm. Despite their simple architecture they are involved in an unprecedented range of metabolic functions that vary with the organism in which they occur. General functions include the β-oxidation of fatty acids and the detoxification of hydrogen peroxide (for a review see [1]).

A characteristic feature of peroxisomes is that they develop in response to external cues. In yeast, peroxisome numbers and enzyme repertoires can be readily prescribed by manipulating the growth substrates ([2]) This, together with the unique property that yeast mutants affected in peroxisome assembly are viable, renders them very attractive model organisms to study the origin, formation and function of peroxisomes. Based on research in yeast various genes essential for peroxisome biogenesis (termed *PEX* genes; Table 1) have been identified and analyzed for their function. However, details on the origin and molecular mechanisms involved in the formation of the organelles are still unresolved.

For long, yeast peroxisomes were considered semi-autonomous organelles that multiply by growth and division of pre-existing ones [3]. Recently, however, a crucial role of the endoplasmic reticulum (ER) in peroxisome formation was proposed thereby challenging the classical growth and division model [4,5]. This model prescribes that all peroxisomal membrane proteins (PMPs) are first sorted to the ER. Two classes of PMPs are subsequently incorporated in two types of biochemically distinct vesicles that subsequently undergo Pex1/Pex6 dependent heterotypic fusion to form peroxisomes [6]. However, in yeast organelle fission appears to represent the dominant mode of organelle multiplication in wild-type cells [7,8].

Detailed analysis of the phenotype of peroxisome-deficient mutants (*pex* mutants) has given important clues on the function of the defective *PEX* gene. However, the deletion of a specific *PEX* gene sometimes leads to different phenotypes in different model organisms, making it difficult to draw conclusions on their function. For instance, cells of *Yarrowia lipolytica PEX19* deletion strains (*pex19*) still contain peroxisomes [9], whereas the corresponding deletion in other species (i.e. baker's yeast and *Hansenula polymorpha*) results in an almost complete lack of these organelles [10,11]. Moreover, also the choice of organelle markers may affect the interpretation of the experimental data. This for instance became clear in *H. polymorpha pex3* mutants in which the receptor docking proteins (Pex13, Pex14) are localized in punctate structures in conjunction with the RING finger proteins (Pex2, Pex10, Pex12) localized to the cytosol [12].

In this contribution we will give an overview on the reported phenotypes of various yeast *pex* mutants. For some yeast *pex* mutants different mutant phenotypes have been reported, which may be due to the use of different marker proteins and experimental procedures. Related to this, we discuss the principles of peroxisome reintroduction in these *pex* mutants as well as the current knowledge on sorting of PMPs and lipids.

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Table 1
Peroxins in yeast and filamentous fungi*.

Matrix protein import	Pex5	PTS1 receptor
	Pex7	PTS2 receptor
	Pex18, Pex20, Pex21	Pex7 co-receptors
	Pex13, Pex14, Pex17, Pex14–Pex17, Pex33	Components of the receptor cocking site
	Pex8	Cargo release, importomer assembly
	Pex22	Anchoring protein for Pex4
	Pex4	Ubiquitin conjugating enzyme involved in receptor ubiquitination
	Pex2, Pex10, Pex12	Components of the ring complex involved in receptor ubiquitination (ligase)
	Pex15, Pex26	Anchoring proteins for Pex1 and Pex6
	Pex1, Pex6	AAA-type ATPases involved in receptor recycling
Regulation of organelle size or abundance	Pex11	Membrane elongation and GTPase activating protein for Dnm1
	Pex23, Pex24, Pex28, Pex29, Pex30, Pex31, Pex32	Form a complex with reticulon homology domain-containing proteins and establish peroxisome contact sites at ER subdomains
	Pex25	Membrane elongation and modeling
	Pex27	Negatively affects fission
	Pex34	Positive regulator of fission
Peroxisomal membrane biogenesis	Pex3	Membrane anchor for Pex19
	Pex19	mPTS receptor

* Adapted from [1].

2. On the origin of peroxisomes

In eukaryotic cells two main categories of cell organelles exist: organelles of the endomembrane system (ER, Golgi apparatus, vacuole) and semi-autonomous organelles (mitochondria, chloroplasts). All membrane compartments that belong to the endomembrane system have their origin in the ER, to which almost all endomembrane proteins are initially sorted and where the bulk of the membrane lipids are synthesized [13]. Trafficking of proteins and lipids to other endomembrane compartments is accomplished by vesicular carriers [14]. Proteins of semi-autonomous organelle are not first transported to the ER but either synthesized inside these organelles or directly imported from the cytosol [15]. Recent studies indicate that membrane lipids are transported to these organelles from other membranes at membrane contact sites (MSC) [16]. Semi-autonomous organelles invariably originate by fission of pre-existing ones.

The origin of peroxisomes is still debated. Consensus exist that peroxisomal matrix proteins are directly imported into peroxisomes upon their synthesis in the cytosol. Also, it is generally accepted that these organelles can divide like mitochondria. However, it has also been suggested that peroxisomes are a branch of the endomembrane system [5]. The latter is predominantly based on observations made in peroxisome reintroduction experiments where *pex* mutants are complemented with the missing genes. Likely, this process of peroxisome formation differs from that occurring under normal conditions in wild-type cells (see below).

Detailed studies using yeast mutants defective in peroxisome fission suggested that new peroxisomes predominantly originate by fission of pre-existing ones in wild-type yeast cells [7,8,17–19]. This model however does not exclude that (a subset of) PMPs first sort to the ER and subsequently are transported via vesicles to pre-existing organelles. In this way peroxisomes may receive their lipids from the ER, where they are predominantly synthesized. Alternatively, PMPs are directly inserted into peroxisomal membranes [20] and lipids transported to these membranes via non-vesicular transport [21].

Below we discuss the current knowledge of *pex* mutants and how peroxisome reintroduction experiments contribute to our understanding of peroxisome formation in wild-type yeast cells.

3. Yeast peroxisome-deficient (*pex*) mutants

Almost three decades ago the first yeast peroxisome-deficient mutants were isolated. Such mutants were viable and capable to grow on glucose, but not on carbon sources that are metabolized by peroxisomal enzymes, such as oleic acid and methanol [22,23]. This property strongly facilitated the isolation of *pex* mutants and the identification of the specific genes involved (termed *PEX* genes) by functional complementation [24]. Later, also other approaches such as organelle proteomics and systems biology resulted in the identification of *PEX* genes [2]. So far, 34 *PEX* genes have been described, which can be divided in three major groups (Table 1).

3.1. PEX genes that control peroxisome size, abundance or dynamics

The least studied *PEX* genes are those whose deletion result in aberrant peroxisome numbers or size (Table 1). Mutants defective in these genes generally do not show defects in peroxisome function, because they are not defective in sorting of PMPs or matrix proteins. The phenotype of such mutants often varies dependent on the organism studied. E.g. while the lack of Pex30 in *Saccharomyces cerevisiae* leads to an increase in the number of normal-sized peroxisomes [25] in *Pichia pastoris* its absence results in the appearance of fewer and clustered peroxisomes [26].

Of this group of peroxins Pex11 is most extensively studied and implicated in peroxisome fission. Deletion of *PEX11* invariably results in a reduction of peroxisome numbers in conjunction with an increase in organellar size (Fig. 1AB). Recent studies revealed that Pex11 both plays a role in the initial organelle elongation [27] as well as in the final organelle scission step [28]. Upon reintroduction of *PEX11* in *pex11* cells it is assumed that newly synthesized Pex11 protein is sorted to the pre-existing organelle, where the protein subsequently mediates normal fission again.

3.2. PEX genes encoding peroxins involved in matrix protein import

PEX genes involved in matrix protein import have been most extensively studied. Mutations in these genes results in mislocalization of peroxisomal matrix proteins, but PMPs are normally inserted into membranes of the predominantly "empty" peroxisomal membrane remnant structures (also designated ghosts) that are still present in these mutants [11,29]. For most peroxins of this category it is known in which stage of the import cycle they function (i.e. recognition of the peroxisomal targeting signal (PTS); receptor docking; receptor recycling). However, the exact molecular function of several of these peroxins is still speculative. Also, the substructure of ghosts in different *pex* mutants defective in matrix protein import varies. Careful electron microscopy analysis of cells of different *H. polymorpha* deletion strains defective in matrix protein import revealed three major morphological classes, namely i) cells that contained virtually normal peroxisomes (*pex7*, *pex17* and *pex20*), ii) cells that contained very small, spherical peroxisomal structures, which harbored very low amounts of matrix protein (*pex2*, *pex4*, *pex5*, *pex10*, *pex12* and *pex14*; Fig. 1D) and iii) mutants that contained multilamellar membrane sheets that lack an apparent proteinaceous matrix (*pex1*, *pex6*, *pex8* and *pex13*; Fig. 1C) [29]. The presence of virtually normal peroxisomes in *pex7* and *pex20* cells can be explained by the fact that in these mutants only import of PTS2 proteins is blocked, which represents a minor portion of all peroxisomal matrix proteins. However, it remains to be analyzed why for instance the deletion of different genes involved in receptor recycling (*PEX2*, *PEX4*, *PEX10*, *PEX12*, *PEX1*, *PEX6*) do not show the same morphological phenotype. Similarly, disruption of different genes encoding genes of

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