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Peroxins: A Proliferation Romance amongst Supposition and Disposition

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ABSTRACT

Since the discovery of peroxisomes over half a century ago, the fundamental mechanism of their biogenesis has remained a matter of debate. The outcome of recent investigations focusing on macromolecular associations coupled with peroxisome formation offers new insight for understanding this process. Peroxisome biogenesis includes the induction and import of membrane and matrix proteins, as well as proliferation and inheritance. When they become superfluous, peroxisomes are rapidly and selectively degraded via pexophagy. Many of the crucial components have been identified in genetic screens. The yeast *Saccharomyces cerevisiae* presents the advantage of being able to grow under conditions in which peroxisomes do not seem necessary such as fermentative growth on glucose media. Physiologically, the survival of a few peroxisomes possibly enables the cell to rapidly respond to new environmental conditions that require the full peroxisomal function. Accordingly, the expression of genes encoding various peroxisomal proteins is repressed by glucose and induced by the presence of fatty acids in the culture medium. Peroxisome biogenesis is controlled by a set of proteins, the peroxins. Most peroxins were originally identified in yeast species. This review aims to discuss the involvement of a range of peroxins in the process of proliferation which is essential to adapt the number of peroxisomes to the cellular needs. Among the known participants, PEX11 is the most prominent and best-studied and its homologues PEX25 and PEX27 have been involved, as well. We also consider the role of the recently identified yeast peroxins namely, PEX28, PEX29, PEX30, PEX31 and PEX32.

Keywords: dysferlin, organelle division, peroxisomes, proliferation

Abbreviations: ARF, ADP ribosylation factor; ***At***, *Arabidopsis thaliana*; ***C. elegans***, *Caenorhabditis elegans*; **CG**, complementation groups; ***H. polymorpha***, *Hansenula polymorpha*; ***Hs***, *Homo sapiens*; **IRD**, infantile Refsum disease; **LDH**, lactate dehydrogenase; **NALD**, neonatal adrenoleukodystrophy; **4-PBA**, 4-phenylbutyrate; **PBD**, peroxisome biogenesis disorders; **PPAR**, peroxisome proliferator activator receptor; **PPRE**, peroxisome proliferator response element; **RCDP1**, rhizomelic chondrodysplasia punctata Type 1; **RNAi**, RNA interference; ***Sc***, *Saccharomyces cerevisiae*; **TPR**, tetratricopeptide repeat; ***Yl***, *Yarrowia lipolytica*; **ZS**, Zellweger syndrome

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INTRODUCTION

Compartmentalization of biochemical pathways is an essential and beneficial trait of eukaryotes. This structural differentiation, however, requires rigorous orchestration and constant adjustments of the cellular content when external conditions change and when cells progress through the cell cycle. Moreover, in their life span cells come upon various adverse situations that could endanger their homeostasis and survival such as changes in nutrient availability, variations in temperature or other stress situations such as being confronted with oxidative agents or xenobiotics. To maintain their metabolic steady-state cells have evolved signaling mechanisms prompting transcriptional reprogramming to achieve a general metabolic remodeling that guides them through adaptation in response to external assaults.

The origin of subcellular organelles has long been a matter of debate. There appear to be two types of organelles, namely the autonomous organelles (endoplasmic reticulum, mitochondria and chloroplasts) and the non-autonomous ones (endosomes, lysosomes, vacuoles and secretory vesicles). It is generally accepted that autonomous organelles multiply by growth and division and therefore cannot be generated *de novo* (Nunnari and Walter 1996; Warren and Wickner 1996), whereas the others all derive from the ER.

The mechanism of peroxisome biogenesis has long been controversial. Peroxisomes were not regarded as typical autonomous organelles nor was there sufficient evidence for

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their descent from the ER. Are they synthesized *de novo*? Do they just grow and divide from preexisting organelles? Or do peroxisomes originate from the combination of both processes? This dilemma only received modest attention from the scientific community who thought that the biogenesis of this single membrane-bounded organelle was a simple variation of that of other organelles. The concepts that evolved during the last decades, however, illustrate that this process rests upon novel sophisticated mechanisms. The development of yeast genetic screens and high throughput proteomic approaches have allowed to identify numerous proteins required for peroxisome biogenesis. Beside our increasing knowledge of the single components and parts of the apparatus involved, the full comprehension of how the molecular machinery of peroxisome biogenesis works is still missing. Functional studies of proteins have been accomplished but how these act together and how this leads to peroxisome division or degradation is mostly enigmatic. All details of the molecular mechanism underlying matrix protein import are still not yet understood and the mechanism of peroxisome proliferation has only started to be unraveled. In this review we summarize the current knowledge about the mechanism by which cells adapt number, size and content of peroxisomes to their environment.

Degradation and synthesis of organelles has to be tightly regulated according to the metabolic status of the cell. This is especially true for multi-purpose organelles such as peroxisomes whose size, number, shape and protein content strongly vary depending on the cell and tissue type as well as on the developmental and physiological state of the organism. Interestingly, all yeast mutant cells affected in peroxisome biogenesis are sensitive to stress assault as demonstrated by their difficulty to grow on media containing high salt concentration compared to wild type (Dunn *et al*. 2004). Such an observation together with the fact that in yeast, peroxisomal functions seem to be required exclusively under stress situations reveals that these organelles may have a vital role in cellular adaptation to stress conditions.

PEROXISOMES AND PEROXINS

Peroxisomes are essential organelles in most eukaryotic cells; they are found in cells as distantly related as protozoa, yeasts, plants and animals (Keller *et al*. 1991). These organelles are surrounded by a single phospholipid bilayer. They are spherical in shape although their size (0.1-1 µm in diameter), number and enzymatic content fluctuate upon the need of the organism, the cell type and the metabolic activities (Subramani 1993; Wanders and Waterham 2006). The importance of peroxisomes for life has been established by the inability of seeds to germinate when peroxisomes are not functional (Lin *et al*. 1999; Hayashi and Nishimura 2003) and by the occurrence of human genetic disorders e.g. Zellweger syndrome or neonatal adrenoleukodystrophy (Moser *et al*. 1995; Wanders 2004).

In comparison, mitochondria are enclosed by a sophisticated double membrane system and harbor more metabolic reactions than peroxisomes. Considering that yeast mitochondria contain 850 proteins (Reinders *et al*. 2006) and mammalian mitochondria supposedly more than 1400 (da Cruz *et al*. 2005) approximately 200 proteins can be expected in peroxisomes although the exact number remains to be determined. Peroxisomes possess the ability to generate and destroy hydrogen peroxide through the activity of their oxidases and catalase, respectively. Indeed, they obtained their name as an illustration of this metabolism. They also metabolize lipids, nitrogen bases and carbohydrates (Lazarow *et al*. 1985; van den Bosch *et al*. 1992; Subramani 1998; Purdue and Lazarow 2001). Growth of some yeast species on methanol (Veenhuis *et al*. 1978, 1981), fatty acids (Veenhuis *et al*. 1987) or alkanes (Kawamoto *et al*. 1978) drastically increases the size and number of peroxisomes. Correspondingly, in the absence of peroxisomes yeast cells are unable to grow on the carbon sources mentioned.

Mutations have been identified in the yeast *S. cerevisiae* (Erdmann *et al*. 1989) that mimic human genetic disorders (Tabak *et al*. 1999) and the corresponding yeast mutants constitute ideal model systems to analyze the molecular mechanism underlying peroxisome biogenesis and proliferation. Altogether the proteins involved in peroxisome biogenesis and proliferation have been coined peroxins and their genes PEX (Distel *et al*. 1996; Kiel *et al*. 2006). A characteristic common to all *pex*-mutants is that they lack functional peroxisomes, which is frequently associated with the mistargeting of peroxisomal matrix proteins. As a consequence some metabolic functions normally enclosed into peroxisomes such as β-oxidation in yeasts are altered in these mutants. Phenotypically, many *pex*-mutants are unable to multiply under conditions that normally lead to peroxisome proliferation e.g. oleic acid induction of yeast cells. Altogether 32 PEX genes from different organisms have been cloned and sequenced (Vizeacoumar *et al*. 2004) but no organism contains all of them. Striking advance has been made in the genetic classification of peroxisomal disorders owing to complementation analysis and homology probing with yeast *PEX* genes. In general, peroxisomal disorders can be divided into two major groups (Moser *et al*. 1995): i) those in which the biogenesis and formation of the organelle is affected resulting in the loss of multiple peroxisomal functions (Peroxisome Biogenesis Disorders, PBD MIM# 601539), which includes Zellweger syndrome (ZS; MIM# 214100), neonatal adrenoleukodystrophy (NALD; MIM# 202370), infantile Refsum disease (IRD; MIM# 266510), and rhizomelic chondrodysplasia punctata Type 1 (RCDP1; MIM# 215100), and ii) those in which one specific peroxisomal protein is deficient but peroxisomes remain intact. PBDs constitute a group of genetically heterogeneous disorders that have been classified into altogether 13 complementation groups (CG) and the genes mutated in all 13 CGs have been identified and sequenced (Eckert and Erdmann 2003; Yan *et al*. 2005; Wanders and Waterham 2006). Although PBDs represent a small group of hereditary diseases these are mostly lethal and no long-lasting therapeutic approaches have been developed yet.

The various peroxins fulfill defined functions, although not all of them have been elucidated at the level of molecular mechanisms. Three peroxins seem to be required for membrane biogenesis and transport of peroxisomal membrane proteins (PEX3, PEX16 and PEX19). PEX3 and PEX19 were shown to be responsible for the sorting of membrane proteins to the peroxisomes. Both of these proteins are conserved in most organisms. In mammalian cells, another peroxin, PEX16 was demonstrated to be required for the *de novo* formation of peroxisomal membranes (South and Gould 1999). Interestingly, this latter peroxin is absent in most yeast species except *Yarrowia lipolytica* where it was demonstrated to be associated with peroxisome proliferation rather than peroxisome biogenesis *per se* (Guo *et al*. 2003). Other peroxins such as PEX5 and PEX7 act as receptors for peroxisomal matrix proteins. While PEX13, PEX14 and PEX17 play a role in the docking of the cargo-loaded receptors, PEX2, PEX8, PEX10 and PEX12 were shown to participate in the recycling of the receptors. Another role was also suggested for PEX14 from studies in the yeast *Hansenula polymorpha* indicating that the phosphorylated form of docking factor PEX14 may be recognized by the degradation machinery (Leao and Kiel 2003). Although such role for PEX14 has not yet been investigated in other organisms a connection between peroxisome formation and degradation would demonstrate that those two mechanisms may be intimately linked. The peroxins known to be involved in proliferation of peroxisomes are PEX11, PEX23, PEX24, PEX25, PEX27, PEX28, PEX29, PEX30, PEX31 and PEX32 although among these ten proteins only PEX11 was found in all organisms, so far (**Table 1**).

While the peroxisome machinery seems to be conserved from yeast to man some subtle differences can be found and some organisms seem to contain several proteins with simi-

Table 1 Known peroxins and correlated peroxisomal disorders.

Gene	Functional orthologs	Identified in			Human gene locus	CG Gifu*		Correlated diseases
		Sc	*Yl*	*Hs*				
PEX1		+	+	+	7q21.q22	1	E	ZS/NALD/IRD
PEX2		+	+	+	8q21.1	10	F	ZS
PEX3		+	+	+	6q23.q24	12	G	NALD
PEX4		+	-	-		-		
PEX5		+	+	+	12q13.3	2	-	ZS/NALD/IRD
PEX6		+	+	+	6q21.1	4 (=6)	C	ZS/NALD/IRD
PEX7		+	+	+	6q21.q22.2	11	R	RCDP
PEX8		+	+	-		-		
PEX9		*Eliminated, wrong ORF***				-		
PEX10		+	+	+	1q36.32	7 (=5)	B	ZS/NALD/IRD
PEX11	PEX25/PEX27	+	+	+	15q25.2 (α) 1q21.1 (β) 19q13.3 (γ)	-		
PEX12		+	+	+	17q21.1	3	-	ZS/NALD/IRD
PEX13		+	-	+	2q14.p16	13	H	ZS/NALD
PEX14		+	+	+	1q36.22	-	K	ZS
PEX15	PEX26	+	-	-		-		
PEX16		-	+	+	11p11.11	9	D	ZS
PEX17		+	-	-		-		
PEX18	PEX20	+	-	-		-		
PEX19		+	+	+	1q22	14	J	ZS
PEX20	PEX18/PEX21	-	+	-		-		
PEX21	PEX20	+	-	-		-		
PEX22		+	-	-		-		
PEX23	PEX30/31/32	-	+	-		-		
PEX24	PEX28/29	-	+	-		-		
PEX25	PEX11	+	-	-		-		
PEX26	PEX15	-	-	+	22q11.21	8	A	ZS/NALD/IRD
PEX27	PEX11	+	-	-		-		
PEX28	PEX24	+	-	-		-		
PEX29	PEX24	+	-	-		-		
PEX30	PEX23	+	-	-		-		
PEX31	PEX23	+	-	-		-		
PEX32	PEX23	+	-	-		-		

CG, complementation group; *Gifu, complementation grouping of Gifu University School of Medicine; ** Kiel *et al*. 2006.

larities to known peroxins reminiscent of gene duplication (Kiel *et al*. 2006). In some organisms distinct peroxins enclose functions that are shared between several factors in others. For instance, the function of the yeast peroxins *Yl*PEX20 or ScPEX18 and *Sc*PEX21 in bringing together the two matrix protein receptors (PEX7 and PEX5) seems to be exclusively achieved by PEX5L, the longer isoform of PEX5 in higher eukaryotes (Dodt *et al*. 2001).

PEROXISOME BIOGENESIS

Biogenesis of peroxisomes includes induction, import of membrane and matrix proteins, proliferation and inheritance. When peroxisomes become superfluous, they are rapidly and selectively degraded via pexophagy (Leao and Kiel 2003; Farre and Subramani 2004). The yeast *S. cerevisiae* presents the advantage of being able to grow under conditions where peroxisomes do not seem necessary such as fermentative growth on glucose media. From morphological analysis in various studies, it can be reasoned that upon glucose growth this yeast contains one or a few small peroxisomes. Peroxisomes do not proliferate when yeast cells grow on glucose as sole carbon source but, during cell division the peroxisome present in each cell divides such that the mother and the daughter cell both enclose one organelle (Fagarasanu *et al*. 2005). Physiologically, the survival of one or few peroxisomes enables the cell to rapidly respond to new variations in the environment that require the function of peroxisomes.

The prevailing hypothesis for peroxisome biogenesis is that they originate from the ER, grow, accumulate proteins and divide at some unknown point to give rise to smaller organelles that yet again accumulate matrix proteins as illustrated in **Fig. 1** (Tabak *et al*. 2003; Kunau 2005; Schluter *et al*. 2006). However, many questions remain unanswered.

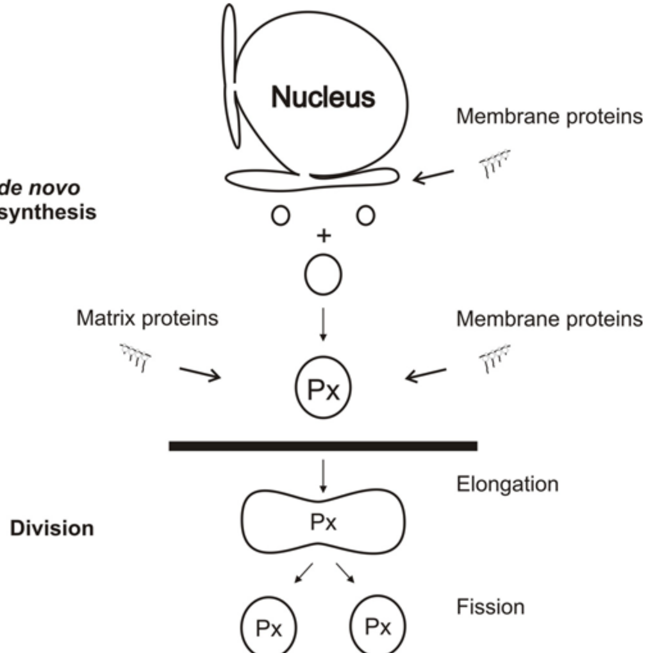

Fig. 1 Model for peroxisome biogenesis. Peroxisomes are synthesized *de novo* or proliferate by division of existing organelles. The model is discussed in the text.

At which time point are proteins translocated into the peroxisomal matrix? Does this occur via vesicular fusion (Titorenko *et al*. 2000) or does membrane invagination occur (McNew and Goodman 1996)? Is there a translocation pore

at the peroxisomal membrane to translocate matrix proteins into the organelle (Erdmann and Schliebs 2005)? By which mechanism and under which stimuli do peroxisomes grow? Are peroxisomes programmed to divide at a determined size or is there some kind of sensing factor signaling them to do so? Are all peroxisomes import-competent? Do peroxisomes have a finite lifespan? The answer to those questions has long been postponed due to technical limitations to study peroxisome biogenesis. Although more than three decades ago electron microscopic pictures of peroxisomes showed that they sometimes presented physical association with the ER (Novikoff and Novikoff 1972), the lack of biochemical connection to ER components has cast some doubt about their true origin. The confirmation came only recently with the results of elaborate real-time fluorescence microscopy analyses in the yeast *S. cerevisiae*, which elegantly demonstrated that PEX3 proteins required for the formation of new peroxisomal membranes accumulate at the ER membrane and recruit PEX19 before the maturation of the peroxisomal membrane (Hoepfner *et al*. 2005). Similarly, using a modified photoactivable version of the green fluorescent protein from *Aequorea victoria* fused with PEX16, Kim *et al*. showed in mammalian cells that PEX16 is inserted into the ER membrane and as such regulates the process of peroxisome *de novo* formation by recruiting other membrane proteins (Kim *et al*. 2006).

In line with these most recent results the ER membrane seems to represent the cradle for peroxisome biogenesis (Hoepfner *et al*. 2005; Kim *et al*. 2006) and, in addition, peroxisomes have the ability to grow and divide by fission (Lazarow and Fujiki 1985). But the question remains as how these two modes of formation are distributed and whether both *de novo* biogenesis and division of already existing peroxisomes are regulated or whether one mechanism is constitutive and the other one regulated. Indeed, how the cell coordinates the overall dynamic turnover of organelles is still an open question. Both processes may not be equally important. While in some organisms *de novo* synthesis might be the most prominent one in others growth and division may prevail.

Peroxisomes are very adaptable organelles. Their number and size can vary in different cell types under normal growth conditions or under stress suggesting that constitutive as well as regulated mechanisms must exist for raising peroxisome abundance beyond one per cell. Peroxisomes respond to environmental stimuli and proliferate or are degraded depending on the need of the cells. A unique feature of peroxisomes is their massive proliferation upon stimulation by herbicides, xenobiotics, ozone or during senescence (Lazarow and Fujiki 1985; Pastori and del Rio 1997) usually associated with increased synthesis of some of their enzymes predominantly those involved in the β-oxidation of fatty acids. In mammalian cells, the expression of genes involved in lipid homeostasis is controlled by the alpha-form of the peroxisome proliferator activator receptor (PPARα). PPARα binds to the peroxisome proliferator response element (PPRE) in the promoters of the regulated genes (Lemberger *et al*. 1996). Ligands have been found for PPARα that include medium or long chain fatty acids as well as hypolipidemic drugs such as fibrates (Issemann and Green 1990). Clofibrate treatment results for instance in amplified expression of PPARα-regulated genes, including the gene coding for acyl-CoA oxidase in rodent hepatocytes. Two studies reported that the activation by PPARα does not affect the expression of *PEX* genes (Okumoto *et al*. 1998; Shimizu *et al*. 1999). The functions assumed by the proteins involved in peroxisome biogenesis are summarized in **Fig. 2** and discussed in detail below.

	H. sapiens	*Y. lipolytica*	*S. cerevisiae*
Membrane Biogenesis	PEX3 PEX16 PEX19	PEX3 PEX16 PEX19	PEX3 PEX19
Elongation	PEX11α PEX11β PEX11γ	PEX11	PEX11 PEX25 PEX27
Fission	DLP1 FIS1	?	VPS, DNM1 FIS1
Number, size, separation	?	PEX24	PEX28 PEX29
		PEX23	PEX30 PEX31 PEX32
		?	RHO1
Inheritance	?	?	INP1 INP2 MYO2

Fig. 2 Proteins controlling peroxisome abundance and inheritance. Proteins whose participation in different steps of peroxisome elongation, division and inheritance has been confirmed are listed. Details of their functions are described in the text.

Factors involved in peroxisome proliferation

PEX11-family members

Among the few proteins that have been implicated in peroxisome proliferation, PEX11 was shown to be directly involved in this process in yeast and mammals (Erdmann and Blobel 1995; Marshall *et al*. 1995; Abe and Fujiki 1998; Abe *et al*. 1998; Schrader *et al*. 1998). Interestingly, the ultrasmall unicellular red alga *Cyanidioschyzon merolae 10D* lacking a PEX11 orthologue contains only one single microbody (Matsuzaki *et al*. 2004) underscoring the significance of PEX11 for peroxisome proliferation.

In mammals, three genes have been identified that code for PEX11α, PEX11β and PEX11γ, respectively. Expression of the genes *PEX11α* and *PEX11γ* is tissue-specific (Shimizu *et al*. 2004). While the latter genes are most prominently expressed in liver, PEX11β is ubiquitously present in the organism (Schrader *et al*. 1998; Li *et al*. 2002b). Interestingly, *PEX11α* was shown to be the only *PEX11* gene whose expression is inducible by fibrates (Schrader *et al.* 1998). A study revealed that 4-phenylbutyrate (4-PBA) could induce peroxisome proliferation and the expression of *PEX11α* (McGuinness *et al*. 2000; Wei *et al*. 2000). It was also suggested that 4-PBA differs from previously described peroxisome proliferators in that it does not act via PPARα and can induce peroxisome proliferation in mammalian cell culture (Li *et al*. 2002a).

Animal models have been generated that lack either *PEX11α* or *PEX11β* (Li *et al*. 2002a, 2002b). The *PEX11β* knockout mice display many of the pathologic characterristics of the known Zellweger syndrome mouse models *PEX5-/-* or *PEX2-/-*, including neuronal migration defect, enhanced neuronal apoptosis, developmental delay, neonatal hypotonia, and neonatal lethality. However, *PEX11β*-deficient mice did not display the peroxisomal enzyme import defects that are the cellular trait of the Zellweger syndrome. In contrast, mice lacking *PEX11α* were indistinguishable from their wild type and heterozygous littermates, they all developed normally. They had no detectable defect in constitutive peroxisome division, and they displayed a normal peroxisome proliferation response to PPARα-activating drugs. On the other hand, overproduction of PEX11α was sufficient to induce peroxisome proliferation in mouse and human cultured cells regardless of the cellular metabolism (Li and Gould 2002). It is however, noteworthy that *PEX11γ* overexpression did not increase peroxisome abundance, but induced tubulation, enlargement, and clustering of peroxisomes (Li *et al*. 2002a).

The rat PEX11α was shown to hold a C-terminal

KXKXX motif typical for ER resident proteins. A peroxisome enriched fraction recruited ARF and coatomer leading the authors to suggest that PEX11 may be involved in binding the coatomer (Passreiter *et al*. 1998). Although this ability was not disputed it was later demonstrated that PEX11 binding to the coatomer is not required for its function in peroxisome proliferation (Maier *et al*. 2000). It is, however, notable that among the three known human PEX11, only PEX11α contains a KXKXX motif in its C-terminal region suggesting the involvement of the coatomer only under particular circumstances, e.g. PEX11α-driven proliferation (Maier *et al*. 2000).

Physiological levels of PEX11 are sufficient to cause fragmentation of peroxisomes and peroxisome fission is inhibited in its absence (Erdmann and Blobel 1995; Voncken *et al*. 2003). PEX11 proteins may influence the overall membrane curvature and associate with specific lipids to determine the correct composition of the peroxisomal membrane. Co-localization studies of heterologously expressed *PEX11* from *Trypanosoma brucei* in mammalian and in yeast cells showed that PEX11 was sorted to peroxisomes in these cells (Lorenz *et al*. 1998), reminiscent of a strong functional conservation. All PEX11 proteins identified share common features, they are small, very basic, and carry an unusually high percentage of hydrophobic amino acids. PEX11 is also the most abundant peroxin at the peroxisomal membrane. Whereas *Sc*PEX11 was suggested to be accessible from the cytosol (Marshall *et al*. 1996) the trypanosome PEX11 and human PEX11γ remained insensitive to digestion by external proteases (Tanaka *et al*. 2003). Although, the nature of PEX11 association with the membrane is still controversial, its topology has great implications for its potential to interact with other cellular components. Sequence analysis reported the existence of a putative membrane targeting signal consensus immediately downstream of the predicted transmembrane residues in *Trypanosoma brucei* (Lorenz *et al*. 1998). This consensus sequence might be responsible for the interaction between the peroxisomal membrane protein receptor PEX19 and PEX11 (Sacksteder *et al*. 2000; Rottensteiner *et al*. 2004; Fransen *et al*. 2005).

In mouse, the PEX11 protein was suggested to indirectly promote peroxisome proliferation by recruiting the dynamin-like protein DLP1 to the peroxisome membrane (Li and Gould 2003), and VPS1 may be similarly recruited in yeast (Hoepfner *et al*. 2001). Analysis of the role of DLP1 in peroxisome proliferation in human cells demonstrated that although PEX11 is required for peroxisome proliferation its presence is not sufficient for the fission process in peroxisome division and that DLP1 is required for this step (Koch *et al*. 2004). Moreover, in a more recent study on the role of microtubules in peroxisome proliferation in fibroblasts from patients with Zellweger syndrome (*pex1*-null cells) over-expression of *PEX11β* could restore the alignment of peroxisomal structures along microtubules as well as binding of DLP1 to these structures but it was not sufficient to promote peroxisome fission (Nguyen *et al*. 2006). Consequently, it can be assumed that PEX11 proteins are involved in the growth of the organelle and that other factors are responsible for the fission event. Even though peroxisomes have been shown to interact with the cytoskeleton (Schrader *et al*. 1996), and class V myosin MYO2 (Hoepfner *et al*. 2001) and Dynein motors (Brocard *et al*. 2005) have been demonstrated to play a role in peroxisome movement and biogenesis, respectively, no peroxisomal protein has yet been identified that directly attach to the cytoskeleton. An essential aspect of peroxisome proliferation may be the release of the organelle from the cytoskeleton, a process in which PEX11 could fulfill a primordial function. It remains to be elucidated whether a communication is established between peroxisomes and the rest of the cell from within the peroxisomal matrix mediated by the activation of PEX11. Other peroxins could play an important role in releasing the organelle from the cytoskeleton.

Plant *pex11*-mutants have recently been characterized and the PEX11 function in plants seems to be conserved (Orth *et al*. 2007). Based on sequence comparison *PEX11* genes were assigned. Studies on peroxisomal dynamics (Mullen *et al*. 2001; Jedd and Chua 2002) and expression data of these *PEX11* genes (Lingard and Trelease 2006; Orth *et al*. 2007) suggest that peroxisomal proliferation mediated by PEX11 takes place in plants like in other organisms. Interestingly, among the five *At*PEX11 proteins identified *At*PEX11-1 (PEX11c), *At*PEX11-2 (PEX11e) and *At*PEX11-5 (PEX11d) carry a C-terminal -KXKXX motif thought to mediate protein retention in the ER and to facilitate the binding of coatomer (Passreiter *et al*. 1998; Andersson *et al*. 1999). Thus, these latter proteins resemble *HsPEX11α* whereas the *At*PEX11-3 (PEX11a) and *At*PEX11-4 (PEX11b) could represent the orthologs of *Hs*PEX11β and HsPEX11γ.

Yeasts differ from higher eukaryotic organisms in that they perform β-oxidation only in peroxisomes. In *S. cerevisiae* the expression of genes encoding many peroxisomal enzymes and few peroxins is repressed by glucose and induced by the presence of fatty acids in the culture medium e.g. oleic acid, among them *Sc*PEX11 and *Sc*PEX25 (Rottensteiner *et al*. 2003a; Rottensteiner *et al*. 2003b; Tam *et al*. 2003). In *S. cerevisiae* oleate-inducible genes contain an oleate-responsive element (ORE) in their promoter sequence that is able to bind the transcriptional activator dimer OAF1/PIP2 (Rottensteiner *et al*. 1997). Disruption of the *Sc*PEX11 gene results in the presence of a giant peroxisome in mutant cells whereas, overexpression of this gene leads to the formation of small peroxisomes in larger amounts than in wild type cells (Erdmann and Blobel 1995). Knowledge of the entire *S. cerevisiae* genome has facilitated the identification of new proteins potentially involved in peroxisome proliferation. Microarray profiling and proteomic approaches have led to the identification of the novel genes *ScPEX25* and *ScPEX27*. The peroxins *Sc*PEX25 (Smith *et al*. 2002) and *Sc*PEX27 (Rottensteiner *et al*. 2003b; Tam *et al*. 2003) together with the dynamin-like protein VPS1 (Hoepfner *et al*. 2001) have been demonstrated to be required for the maintenance of peroxisome size and number in yeast. The similarities in sequence and function suggest that the yeast PEX11-family members are also involved in enlargement and growth of peroxisomes rather than in the fission process.

Dysferlin-containing proteins and membrane proliferation

A number of peroxins, *Yl*PEX23, *Yl*PEX24 and their homologs *Sc*PEX28, *Sc*PEX29, *Sc*PEX30, *Sc*PEX31, *Sc*PEX32 contain dysferlin domains. These new peroxins are peroxisome integral membrane proteins that have an important role in controlling the size and number of the organelle (Yan *et al*. 2005). A dysferlin encoding sequence was first identified as a gene mutated in limb-girdle muscular dystrophy (type 2B) and Miyoshi myopathy (Bashir *et al*. 1998; Liu *et al*. 1998). Since they generate force by contraction skeletal muscle cells are very susceptible to plasma membrane injuries (Alderton and Steinhardt 2000; McNeil and Steinhardt 2003). Evidence from studies of dysferlin-null mice suggests a function for dysferlin in membrane repair (Bansal *et al*. 2003).

The mammalian gene encoding dysferlin shows homology to the Fer-1 gene of *Caenorhabditis elegans* (Bashir *et al*. 1998). Fer-1 is a spermatogenesis factor specifically expressed in primary spermatocytes in *C. elegans*. In spermatids, mutations in Fer-1 cause infertility by impairing fusion of large vesicles called membranous organelles with the plasma membrane (Achanzar and Ward 1997). This fusion event leads to addition of membrane material to the plasma membrane at the fusion site, a process necessary for the extension of the pseudopodia responsible for crawling of the spermatids. Consequently, mutations in Fer-1 lead to immobile spermatids and sterility in *C. elegans* (Achanzar and Ward 1997). Because Dysferlin and Fer-1 contain structural

as well as sequence similarities, it was proposed that dysferlin may also be a vesicle-associated membrane protein involved in the docking and fusion of vesicles in skeletal muscle cells. Following the identification of dysferlin, several other homologous proteins were identified establishing the ferlin-family (Yasunaga *et al*. 1999; Britton *et al*. 2000).

Motif repeats called dysferlin domains (Dysf) with no known function have first been identified in dysferlin. The Dysf domains are commonly described in two parts, the N-terminal region (Dysf-N) and the C-terminal region (Dysf-C). Among Dysf-containing proteins, several integral membrane proteins implicated in the peroxisome proliferation have been identified in yeast. The peroxins PEX23 and PEX24 were first discovered in the yeast *Y. lipolityca* in a screen for mutant cells unable to utilize oleic acid as a sole carbon source (Brown *et al*. 2000; Tam and Rachubinski 2002). Homology probing with *Yl*PEX23 and *Yl*PEX24 soon led to the finding of *S. cerevisiae* homologues *Sc*PEX30, *Sc*PEX31, *Sc*PEX32 (Vizeacoumar *et al*. 2004) and *Sc*PEX28 and *Sc*PEX29 (Vizeacoumar *et al*. 2003), respectively. All these proteins localize to the peroxisomal membrane. Although doomed to confusion, the common structural domain present in all these proteins has been called PEX24 domain in the PFAM database (Tam and Rachubinski 2002) and its function remains entirely unknown. Sequence alignments show conserved blocks of homology through *Yl*PEX23, *Yl*PEX24, *Sc*PEX28, *Sc*PEX29, *Sc*PEX30, *Sc*PEX31 and *Sc*PEX32 protein sequences. Indeed, all members of this protein family contain at least one transmembrane domain and a dysferlin domain as common structural motifs. In line with the role of Fer-1 in membrane fusion (Achanzar and Ward 1997) it would be tempting to speculate that in a manner similar to Fer-1, dysferlin or myoferlin, the five *S. cerevisiae* dysferlin-containing peroxins either alone or assembled may illustrate a role in the attraction of dysferlin-domain protein containing vesicles to the peroxisomal membrane. In support of this hypothesis, it has been noted that mutants in *YlPEX23* accumulate vesicles containing peroxisomal matrix and membrane proteins (Brown *et al*. 2000). For one of the *S. cerevisiae* homolog some interaction partners are already known. ScPEX30 has been recently reported to interact with the peroxin PEX19 believed to be a main player in the translocation of peroxisomal membrane proteins (PMP) and with the small GTPase RHO1 (Yan *et al*. 2005; Vizeacoumar *et al*. 2006) implicated in the regulation of peroxisome membrane dynamics and biogenesis (Marelli *et al*. 2004). Interestingly, PEX30 was shown to interact with PEX29 and PEX31 in a partial two-hybrid screen. Moreover, *Sc*PEX30 is already present in membrane vesicles in non-induced cells (unpublished data) and induced upon growth on oleate (Vizeacoumar *et al*. 2004).

Although yeasts mutated in the PMP encoding genes *PEX30*, *PEX31* or *PEX32* do not present a complete peroxisomal defect, peroxisome proliferation is altered in these cells. Especially, cells lacking PEX30 or all three proteins present an increased number of peroxisomes (Vizeacoumar *et al*. 2004). From mutant analysis it has been suggested that PEX30 may play a role in the regulated control of peroxisome number whereas PEX31 and PEX32 would be mainly implicated in the regulation of peroxisome size. Moreover, these three peroxins may act downstream of PEX28 and PEX29 also demonstrated to regulate peroxisome proliferation (Vizeacoumar *et al*. 2004). Whether these proteins are all members of one macromolecular complex is unknown. Sequence analysis reports show that PEX30, PEX31 and PEX32 contain 2, 4 and 6 hydrophobic regions, respectively, that are putative transmembrane domains. The C-terminal ends of PEX29- and PEX30-tagged fusion proteins were accessible to antibodies when peroxisomes were intact, which demonstrate that this part of the proteins may face the cytosol (unpublished data).

The expression of PEX30 and PEX32 was strongly influenced by the carbon sources. Under conditions when peroxisome function is not required such as glucose growth yeast cells do express PEX30 but its expression is strongly increased when peroxisome proliferation is induced with oleic acid. The expression of PEX32 is only detectable upon induction. In contrast, PEX31 expression seems to be constitutive regardless of whether peroxisome proliferation is induced or not. In summary, the expression of PEX30 and PEX32 is transcriptionally regulated presumably coordinated by the metabolic needs of the cell and may rather be implicated in the negative control of peroxisomal number and size, respectively. In contrast PEX31 is constitutively expressed and may be involved in the down-regulation of peroxisome abundance. However, the down-regulation by PEX31 may be subjected to a different control mechanism. This fits well with the observation that PEX31 was found as a target for the phosphorylation/dephosphorylation regulatory circuit by the protein kinase IPL1 and phosphatase GLC7 (Pinsky *et al*. 2006). Identified in a multicopy suppressor screen of temperature sensitive *Ipl1*-mutant yeasts the PEX31 protein was shown to interact with GLC7. The ILP1 kinase belongs to the Aurora family of kinases, important regulators of chromosome attachment to microtubules (Kotwaliwale and Biggins 2006). It has been suggested that activity of the phosphatase GLC7 guarantees accurate chromosome segregation during cell division by dephosphorylating ILP1 targets rather than regulating the ILP1 kinase. Most likely, PEX31 counteracts the regulatory action of the kinase IPL1 by stimulating GLC7. Alternatively, PEX31 is substrate for both ILP1 and GLC7 and overproduction of PEX31 results in active unmodified PEX31 that acts to release proteins from the microtubules. Whether such a regulatory circuit may be involved in peroxisome biogenesis or inheritance remains to be determined.

The genes *PEX28* and *PEX29* seem to be constitutively expressed upon glucose and oleate growth. Yeast cells lacking PEX28 or/and PEX29 still contain peroxisomes but their proliferation seems to be altered. The absence of PEX29, PEX28 or both leads to an increased number of small peroxisomes, that exhibit extensive clustering, and often display membrane thickening between adjacent peroxisomes in a cluster (Vizeacoumar *et al*. 2003). Thereafter, these new proteins were suggested to participate in the separation of peroxisomes during the proliferation process. Interestingly, although yeast cells deleted in *PEX28* or *PEX29* show no growth retardation on oleate, yeast cells deleted in *PEX29* present a severe growth defect on lactate. Lactate is one of the major carbon sources in natural environments. Therefore, PEX29 could play a role in aerobic metabolism and it could be required for the function of the enzyme lactate dehydrogenase (LDH) converting lactate into pyruvate usually inside the mitochondria. This role could include i) regulation of LDH localization, ii) regulation of the coenzyme (NAD^+) accessibility or iii) involvement in NADH reoxidation. Alternatively, the phenotype observed in *pex29*-mutant cells may be due to a reduced peroxisome proliferation with subsequent metabolic consequences on mitochondria.

To this end, it is noteworthy that the import of peroxisomal matrix proteins is defective in *Y. lipolytica* mutant-cells lacking PEX23 or PEX24 whereas the peroxisomal protein transport is unaffected in *S. cerevisiae* upon deletion of the respective homologs (Brown *et al*. 2000; Tam and Rachubinski 2002; Vizeacoumar *et al*. 2003, 2004). This suggests that although the newly identified peroxins from *S. cerevisiae* contain structural homology to *Yl*PEX23 and *Yl*PEX24 they may assume a different function in peroxisome proliferation. Alternatively, variation in the mechanism of peroxisome biogenesis and proliferation may be a species-specific theme. The yeast *Y. lipolytica* has already been shown to escape the typical yeast mechanism for peroxisome biogenesis since it requires the action of the factor PEX16, which does not seem to exist in *S. cerevisiae*.

Mitochondrial division

Maintenance in number, morphology and distribution of organelles is sensitive to environmental signals and preserved through the balance between fusion and fission, two tightly regulated processes. For instance, mitochondrial fusion and fission are both controlled via GTPases. The fusion of yeast mitochondria requires the two factors FZO1 and MGM1 (Rapaport *et al*. 1998; Wong *et al*. 2003), and mitofusins 1, 2 and OPA1 act as their counterparts in mammalian cells (Eura *et al*. 2003; Cipolat *et al*. 2004). Fission of mitochondria is initiated by the interaction of the large dynamin related GTPase DLP1 with FIS1, a TPR motifs containing-protein (Mozdy *et al*. 2000). The *S. cerevisiae* ortholog DNM1 requires the additional factor MDV1, a WD40 protein serving as adaptor molecule (Tieu *et al*. 2002). A number of other proteins have been identified that exhibit a function in regulating mitochondrial distribution and morphology (Dimmer *et al*. 2002).

The 17kD protein FIS1 is tail-anchored into the outer mitochondrial membrane with its N-terminus facing the cytosol (Stojanovski *et al*. 2003). It is targeted to mitochondria via its C-terminal end (Koch *et al*. 2005). Overexpression of FIS1 in mammalian cells leads to mitochondrial fragmentation and aggregation whereas knockdown via RNAi results in mitochondrial morphological defects and extension of mitochondrial tubules (Stojanovski *et al*. 2003). The *fis1*-mutant yeasts exhibit tubular mitochondrial structures spread out through the whole cytosol thought to be the consequence of an aberrant mitochondrial fission (Mozdy *et al*. 2000). Similarly, cells from the plant *Arabidopsis thaliana* lacking the FIS1-orthologue BIGYIN contain fewer but larger mitochondria (Scott *et al*. 2006). FIS1 is essential to recruit the GTPase DLP/DNM1, to the outer mitochondrial membrane. FIS1 works either alone in mammalian cells or in association with MDV1 in yeast. Only 3% of the whole cellular DLP1 are located to the outer mitochondrial membrane, the majority of molecules being found in the cytosol (Smirnova *et al*. 2001). The TPR region of *Hs*FIS1 was demonstrated to be involved in the interaction with DLP1 (Yu *et al*. 2005). The TPR-like helix bundle of FIS1 (Suzuki *et al*. 2003) extending into the cytosol may be capable of binding the C-terminal ends of DLP1 although the part of DLP1 recognized by FIS1 has not yet been identified. In support of this hypothesis, numerous examples exist for which TPR domains recognize short C-terminal stretches of amino acids, e.g. PEX5 (Brocard *et al*. 1994; Lametschwandtner *et al*. 1998), HOP (Scheufler *et al*. 2000), or APC3 (Vodermaier *et al*. 2003). FIS1 was proposed to either act as recruitment factor for constituents of the mitochondrial division machinery or as a signaling molecule from within the mitochondria. With FIS1 being evenly distributed along the mitochondrial surface it could exert its function in either of two ways: i) the interaction with DLP1 is of transient nature or ii) only a portion of FIS1 that has been previously activated interacts with DLP1 (van der Bliek 2000). A recent model for yeast mitochondrial fission suggests that the MDV1-FIS1 complex recruits DNM1-dimers at the outer mitochondrial membrane (Bhar *et al*. 2006). Once associated with the outer mitochondrial membrane the dimers assemble into multimeric complexes that as a result lead to the reorganization of MDV1 molecules into fission complexes. MDV1 is usually evenly distributed on the outer mitochondrial membrane and only in the lead of this reorganization the protein accumulates in punctate structures that also colocalize with FIS1-containing fission complexes. However, FIS1 itself remains evenly distributed along the mitochondrial outer membrane.

The GTPase DNM1/DLP1 was demonstrated to promote programmed cell death following treatment with various death stimuli (Fannjiang *et al*. 2004). In agreement, prevention of fission events by expression inhibition of DLP1, FIS1 or the adaptor MDV1 in yeast leads to a delay in apoptosis (Lee *et al*. 2004). Obviously, mitochondrial fission is a prerequisite for apoptosis.

MDV1, a so far yeast-specific component of the mitochondrial division apparatus, harbors seven WD40 repeats known to form a beta propeller structure usually involved in protein-protein interaction (Neer *et al*. 1994). At least two interaction partners are known for MDV1 namely, DNM1 (Tieu and Nunnari 2000) and FIS1 (Karren *et al*. 2005). Furthermore, another WD40 repeat protein, CAF4, was recently identified as a component of the mitochondrial division machinery that interacts with FIS1, MDV1 and DNM1 and apparently functions MDV1 alike (Griffin *et al*. 2005) making WD40 a potential structural requirement for mitochondrial fission.

Common elements in mitochondrial and peroxisomal division

Evidence has been found that components of the mitochondrial division apparatus also act on peroxisomes (Koch *et al*. 2003; Li and Gould 2003; Koch *et al*. 2005). For both organelles, proliferation relies on the elongation, constriction and finally division of membranes surrounding a proteinaceous content different from the cytosolic environment. Yet, the mitochondrial division machinery is expected to be more sophisticated since the fission event has to be coordinated between two membrane systems.

The mammalian DLP1 protein was not only localized to mitochondria but also to peroxisomes where it has been suggested to play an essential role in the process of peroxisome division (Koch *et al*. 2003; Li and Gould 2003). Comparable with its localization at constriction sites of the outer mitochondrial membrane DLP1 was also localized at spots along elongated peroxisomes and at the tips of peroxisomal tubules (Koch *et al*. 2003). Silencing DLP1 expression resulted in the reduction of peroxisome abundance and formation of tubular peroxisomes and mitochondria (Koch *et al*. 2003; Li and Gould 2003; Koch *et al*. 2005). Since the tubular extensions of peroxisomes observed in the absence of DLP1 presented constrictions the role of DLP1 was proposed to be restricted to the fission process. Elongation and constriction of peroxisomes may therefore occur independently of DLP1 (Koch *et al*. 2004). When DLP1 silencing was combined with the overexpression of *PEX11β* the formation of tubular membranes with constrictions was strongly enhanced suggesting that in the absence of DLP1 the membrane division process is initiated but not completed (Koch *et al*. 2004). Peroxisome division is seen as a multistep process including elongation, constriction and fission of the organelles. Distinct sets of proteins may be required for these processes. Whereas no key player has yet been characterized to be involved in the process of membrane constriction during peroxisome proliferation (Koch *et al*. 2005), PEX11 is assumed to be involved in elongation and DLP1 is a key component for fission of peroxisomes and mitochondria.

Since organellar fission occurs in all eukaryotes functional homolog must exist for the major players. Accordingly, DRP3A has been identified as DLP1 homolog in plants (Mano *et al*. 2004). The closest ortholog in the yeast *S. cerevisiae* DNM1 is not required for the regulation of peroxisome abundance under non-inducing growth conditions (Hoepfner *et al*. 2001; Kuravi *et al*. 2006). Indeed, in yeast another dynamin related protein, VPS1, identified in purified peroxisome fractions is involved in peroxisome division under all growth conditions (Hoepfner *et al*. 2001; Marelli *et al*. 2004). Its association with peroxisomes is PEX19-dependent (Vizeacoumar *et al*. 2006). *Vps1*-mutant cells grown on glucose exhibit only one or two large peroxisomes that may form long tubules (Hoepfner *et al*. 2001) suggesting a role for VPS1 in peroxisome fission. However, recent observations in *S. cerevisiae* describe the presence of DNM1 on peroxisomes and quantitative fluorescence measurements demonstrate that it participates in the regulation of peroxisome abundance when cells are induced with oleate (Kuravi *et al*. 2006). The lack of VPS1 leads to a re-

duction of peroxisome number under all growth conditions, the absence of DNM1, in contrast, reduces the number of peroxisomes only when cells are grown on oleate. Yeast cells deleted for both dynamin-related proteins usually contain only one but enlarged peroxisome (Kuravi *et al*. 2006).

FIS1 represents another example of a protein with dual localization that is involved in organelle division (Koch *et al*. 2005; Kuravi *et al*. 2006). The last 26 amino acids consisting of a transmembrane domain and a C-terminal tail are sufficient for targeting to both mitochondria and peroxisomes (Koch *et al*. 2005). In human cells co-expression of *FIS1* and *PEX11β* changes the peroxisome distribution. Peroxisomes accumulate in a juxtanuclear position and associate with aggregated/fragmented mitochondria (Koch *et al*. 2005). While over-expression of each protein alone did not alter the uniform distribution of peroxisomes inside the cell it had the same consequence on peroxisome morphology namely, a tubulo-reticular appearance. Therefore it seemed reasonable to assume that PEX11β and FIS1 act together on peroxisome growth and division. Interestingly, a physical interaction between PEX11β and FIS1 could not be demonstrated (Li and Gould 2003; Koch *et al*. 2005). DLP1 is the only binding partner found for *Hs*FIS1 (Yoon *et al*. 2003). Silencing of *FIS1* induces elongation of peroxisomes whereas overexpression of the human *FIS1* leads to an increase in peroxisome fission, which can be suppressed by inhibition of DLP1 indicating a role for both proteins in peroxisome division. Obviously, FIS1 plays similar roles in the division of mitochondria and peroxisomes by either facilitating the targeting of cytosolic DLP1 to membranes or by activating DLP1. Both models would convincingly explain why only the overexpression of *FIS1* and not of *DLP1* enhances peroxisome fission, and why the absence of either protein leads to a similar loss of fission phenotype. In yeast, FIS1 is thought to recruit MDV1 and together these two proteins enroll DNM1 to mitochondrial fission sites (Naylor *et al*. 2006). FIS1 was demonstrated to have a dual location on mitochondria and peroxisomes (Kuravi *et al*. 2006), but an involvement of MDV1 or its homolog CAF4 in the process of peroxisome division in yeast has not yet been revealed.

Obviously, different organelles use the same components for division. VPS1 was originally identified as a protein involved in vacuolar protein sorting and localized to the Golgi (Vater *et al*. 1992), but now it is rather thought to assume its function both at the vacuole (Peters *et al*. 2004) and at peroxisomes (Hoepfner *et al*. 2001; Marelli *et al*. 2004). DLP1/DNM1 is involved in mitochondrial morphology and division (Bleazard *et al*. 1999). In addition to mitochondria the mammalian DLP1 has been localized to the perinuclear region (Imoto *et al*. 1998), to cytoplasmic vesicles, to tubules of the ER (Yoon *et al*. 1998), and to the Golgi apparatus (Koch *et al*. 2004) as well as to peroxisomes (Koch *et al*. 2003; Li and Gould 2003). The yeast protein has also been localized to peroxisomes (Kuravi *et al*. 2006). FIS1 responsible for targeting or activation of DLP1 has been localized to two different organelles, mitochondria and peroxisomes. Clearly, these proteins fulfill their tasks at different membranous systems. However, to maintain their specificity the shared components of the membrane division machineries are most likely interacting with organelle-specific factors that have not yet been identified. It may well be that among the dysferlin-containing peroxins one or the other interacts with members of the general division machinery thus serving as organelle-specific bridging protein.

Proteins involved in peroxisome inheritance

INP1 has been characterized as a peripheral membrane protein of peroxisomes in the yeast *S. cerevisiae*. In its absence, dividing mother cells completely lose their peroxisomes, which all migrate to the daughter cells. In contrast, overexpression of *INP1* resulted in the appearance of immobilized peroxisomes in mother cells that were unable to migrate to the bud. *In vitro* experiments demonstrated that INP1 could interact with proteins known to control peroxisome division, namely PEX25, PEX30 and VPS1 (Fagarasanu *et al*. 2005).

Eukaryotic cells need to faithfully bequeath organelles to their progeny to maintain the benefits of compartmentalization of biochemical pathways. During cell division in *S. cerevisiae* peroxisomes are driven along actin cables to the bud neck by the myosin V motor protein MYO2. The globular tail of MYO2 interacts with the newly identified yeast peroxisomal membrane protein INP2, which has subsequently been suggested to act as peroxisome-specific receptor linking peroxisomes to factors required for their movement (Fagarasanu *et al*. 2006). A model for peroxisome division and inheritance in yeasts is depicted in **Fig. 3**.

The small GTPase RHO1 has been identified at the peroxisomal membrane in yeast. It has been shown *in vitro* that

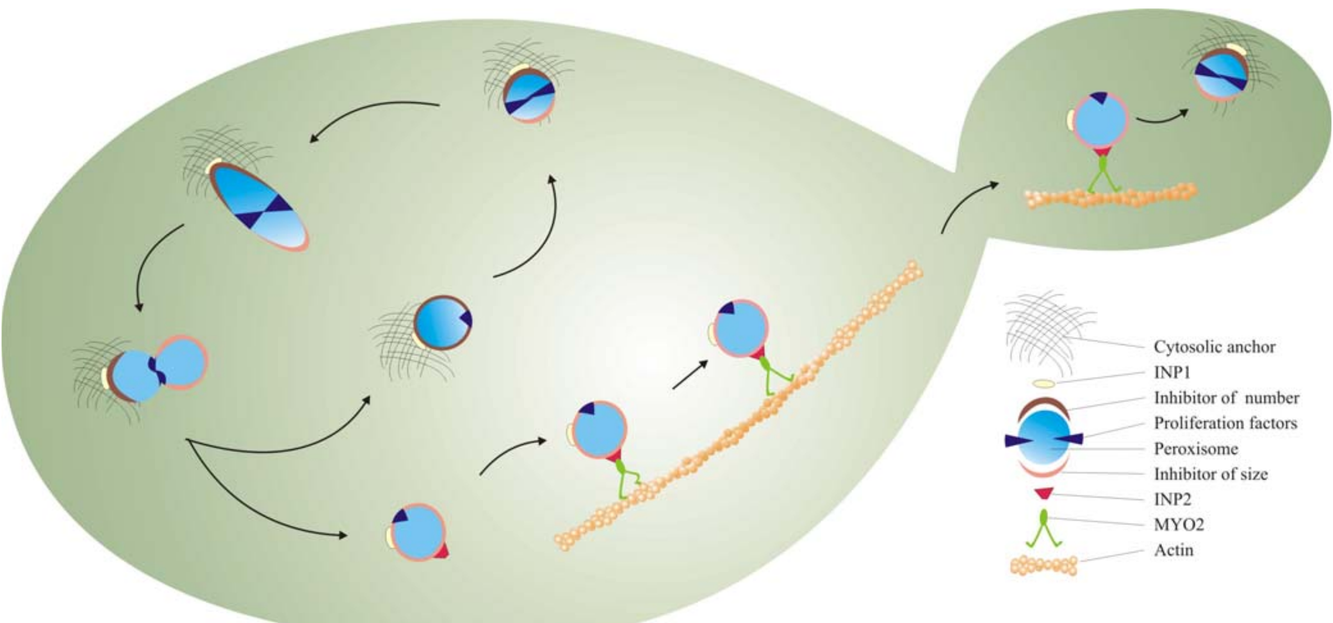

Fig. 3 Model for the proliferation and inheritance of peroxisomes. Peroxisomes are attached to the cytoskeleton via cytosolic anchor proteins and INP1. Factors controlling size and number are dispersed on the surface of peroxisomes exerting their control. Unknown signaling molecules activate the proliferation machinery already present on the peroxisomes finally leading to peroxisome fission. During cell division, selected peroxisomes become associated with MYO2 via INP2 and move along actin cables to the bud.

PEX25 and PEX30 could bind RHO1 suggesting a role for RHO1 in the regulation of peroxisome abundance. In addition, localization studies in mutant cells showed that the presence of PEX25 is required for the recruitment of RHO1 to the peroxisomal membrane where it seems to specifically participate in the reorganization of actin on peroxisomes upon induction (Marelli *et al*. 2004). Therefore, the function of RHO1 has been proposed to be associated with peroxisome membrane dynamics and biogenesis.

It is interesting to note that the two p24 proteins EMP24 and ERP3 have recently been implicated in peroxisome development of the yeast *H. polymorpha* (Otzen *et al*. 2007). Indeed, p24 proteins play a major role in vesicular trafficking and are assumed to connect the coat components to the corresponding cargo proteins (Bremser *et al*. 1999).

CONCLUDING REMARKS

Obviously, the process of organelle division and inheritance represents a further illustration of the well-known principle that eukaryotic cells use the same evolutionary successful system to assume different tasks in which the addition of adaptor modules establishes specificity. For instance, the movement of organelles requires motor proteins and organelle specific interaction partners. Building blocks are used like manufactured units and assembled into the right context. In this particular case the core unit of membrane fission, DLP1/DNM1 and FIS1, is adapted to various systems including peroxisomes. This core unit does not resemble the prokaryotic division apparatus. Bacterial division is initiated by the assembly of the prokaryotic tubulin homolog FTSZ into a ring at the future site of cell division (Romberg and Levin 2003). Several membrane-associated division proteins are recruited to this ring to form a complex, which causes invagination of the cell envelop layers to form a division septum (Harry *et al*. 2006). To compensate for the loss of the envelope eukaryotes have developed a new molecular machine for organelle division.

In view of the growing number of factors identified to participate in the regulation of peroxisome abundance the new focus should be to characterize how these factors act together, to define their interaction with the peroxisomal membrane via lipid-binding proteins, modifying enzymes, and cytoskeletal components, to analyze the spatio-temporal dynamics of peroxisomal membrane protein complex formation and how these factors are regulated during the process of peroxisome proliferation and to study how their dysfunction influences the development of human diseases.

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