CMLS, Cell. Mol. Life Sci. 59 (2002) 1058–1069
1420-682X/02/061058-12 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Human Genome and Diseases: Review

Cellular and molecular aspects of Zellweger syndrome and other peroxisome biogenesis disorders

U. Brosius and J. Gärtner*

Department of Pediatrics, Heinrich Heine University Düsseldorf, Moorenstrasse 5, 40225 Düsseldorf (Germany), Fax + 49 211 8118757, e-mail: gaertnj@uni-duesseldorf.de

Received 16 October 2001; received after revision 2 January 2002; accepted 3 January 2002

Abstract. Peroxisomes are single-membrane-bound organelles present in virtually all eukaryotic cells. They are involved in numerous metabolic processes, both catabolic and anabolic, including β-oxidation of very long chain fatty acids, metabolism of hydrogen peroxide, plasmalogen biosynthesis and bile acid synthesis. In several genetic diseases, there is either isolated deficiency of a specific peroxisomal protein (single-protein deficiencies) or a defect in the formation of the organelle with loss of multiple peroxisomal functions (peroxisome biogenesis disorders). X-linked adrenoleukodystrophy is an example of the former, and the Zellweger spectrum of the latter. Peroxisome biogenesis disorders are inherited in an autosomal recessive manner and result from mutations in any of at least 12 *PEX* genes that encode peroxins. This article reviews the peroxisomal system, the clinical, biochemical and molecular aspects of peroxisomal disorders, and discusses recent scientific advances in the understanding of peroxisome biogenesis.

Key words. Peroxin; *PEX* gene; peroxisome; peroxisome biogenesis; peroxisome biogenesis disorder; Zellweger syndrome.

Peroxisomal system: components and metabolic functions

Peroxisomes are multifunctional organelles present in nearly all eukaryotic cells. They are delimited by a single membrane which is impermeable to protons and small metabolites, creating an enzymatically and chemically unique microenvironment within the cell [1]. Together with glyoxysomes of plants and glycosomes of trypanosomes, they constitute the microbody family. Peroxisomes contain at least one hydrogen-peroxide-producing oxidase and catalase to decompose the hydrogen peroxide [2]. Their morphology is usually spherical in transmission electron micrographs (fig. 1), and they range in di-

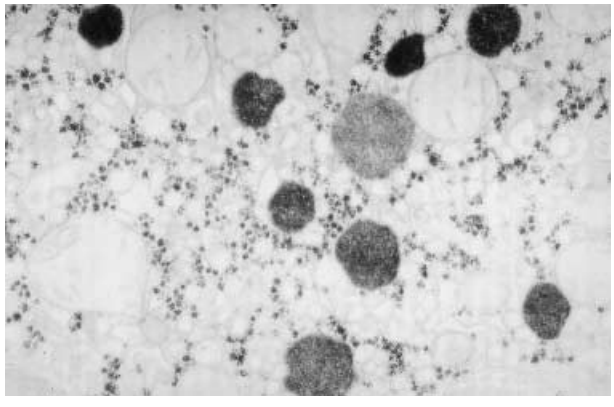


Figure 1. Electron micrograph of normal peroxisomes in human infant liver visualized by diaminobenzidine staining for catalase. The staining intensity is typically heterogeneous (from M. Espeel and F. Roels, University of Gent, Belgium).

* Corresponding author.

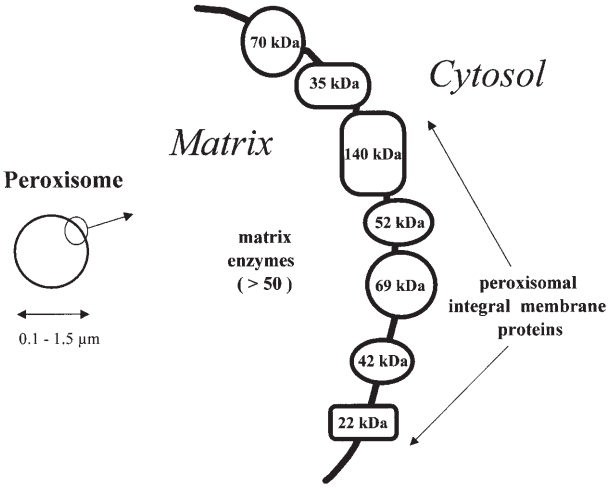


Figure 2. Scheme of the peroxisomal system. Peroxisomes can vary in diameter from 0.1 to 1.5 µm and are usually round in shape. The organelle membrane contains organelle-specific membrane proteins. The peroxisome interior is a protein-rich environment containing more than 50 enzymes for numerous metabolic processes.

ameter from 0.1 to 1.5 µm [3, 4]. Human cells contain several hundred peroxisomes but the number per cell varies depending on the type of cell and its metabolic state [5]. The organelle membrane includes a fine granular matrix and occasionally a paracrystalline core. All peroxisomal matrix and membrane proteins are encoded by nuclear genes, synthesized on free cytosolic polysomes, and posttranslationally imported into peroxisomes [2, 6–8]. The peroxisomal membrane contains more than ten organelle-specific integral membrane proteins of different sizes (fig. 2). The peroxisomal matrix contains more than 50 different proteins, mainly enzymes essential for various metabolic processes (table 1) [9]. Examples are metabolism of hydrogen peroxide, β-oxidation of very long chain fatty acids, plasmalogen biosynthesis and bile acid synthesis.

Peroxisome biogenesis

Biogenesis of peroxisomes requires the formation of a lipid bilayer, the import of peroxisomal membrane proteins into that bilayer, and the transport of proteins and other peroxisomal matrix components across the organelle membrane. Among different organisms in which this process has been studied so far, 24 proteins have been identified to play a role in peroxisome biogenesis. These proteins are called peroxins and *PEX* genes encode them [10].

Different views of organelle assembly

In general, organelle biosynthesis follows either of three distinct models: (i) de novo synthesis, (ii) growth and division of pre-existing organelles or (iii) assembly by participation of other organelle systems [11, 12]. Mitochondrial biogenesis involves repeated rounds of mitochondrial growth and division and seems to be relatively independent of other cell compartments [12]. In contrast, the Golgi apparatus is a complex system of vesicle fusion and depends upon the continuous supply of proteins and lipids by vesicular transport from the endoplasmic reticulum [11, 13, 14]. For peroxisomes, there is little agreement on which of the three models for organelle assembly best explains their biogenesis mechanisms [15–19].

Table 1. Metabolic functions of peroxisomes in different organisms.

Metabolic function		Organisms
Anabolic		
Biosynthesis	ether phospholipids	mammals
	cholesterol	mammals
	bile acids	mammals
	polyunsaturated fatty acids	mammals
	penicillin	fungi
	lysine	yeasts
Catabolic		
Degradation	hydrogen peroxide	yeasts, fungi, plants, mammals
	fatty acids (β-oxidation)	yeasts, fungi, plants, mammals
	fatty acids (α-oxidation)	mammals
	amino acids	yeasts, mammals
	purines	plants, mammals
	polyamines	mammals
	methanol	yeasts
Photorespiration	conversion of serine to glycerate	plants
	conversion of glycolate to glycine	plants
Glyoxylate cycle		yeasts, fungi, plants

The current main model for peroxisome biogenesis proposes that the organelle arises by growth and fission of pre-existing peroxisomes [2]. Peroxisomal membrane and matrix proteins are posttranslationally delivered to the growing organelle, followed by division once a critical size is reached. In contrast, there are other models that propose a de novo biogenesis of peroxisomes [20] and several recent studies have shown that peroxisomes can be synthesized in the absence of pre-existing peroxisomes [21–24]. In addition, earlier studies favored a third model involving the endoplasmic reticulum. These studies found peroxisome membranes spatially associated with the endoplasmic reticulum [25]. Furthermore, studies in yeast demonstrated that mutants defective in protein secretion are also defective in peroxisome biogenesis and that under these conditions, two peroxisomal membrane proteins, Pex2p and Pex16p, accumulated in the endoplasmic reticulum [26]. Although these three models seem to be contradictory, they can be incorporated into one hypothesis [8]: peroxisomes arise either by growth and division of existing peroxisomes or de novo. The growth of existing peroxisomes and the reappearance of peroxisomes in cells lacking the organelle require the posttranslational uptake of membrane and matrix proteins as well as the uptake of peroxisomal proteins and lipid vesicles originating from the endoplasmic reticulum or other endomembranes.

Membrane synthesis

The process of peroxisome membrane synthesis is poorly understood. It includes the formation of lipid bilayers and the integration of peroxisomal membrane proteins. Three peroxins, namely Pex3p, Pex16p and Pex19p, have been identified to play a role in the synthesis of peroxisome membranes de novo [21–24]. However, whether they function in lipid bilayer assembly or in peroxisomal membrane protein targeting and integration is unclear.

The peroxisomal targeting signals for membrane proteins (mPTS) are distinct from those described for matrix proteins. Single targeting signals have been described either in the amino-terminal part, as for Pex3p, Pex14p, Pex22p, PMP22 and PMP70 [24, 27–31], or in the middle part, as for PMP47 and PMP34 [32, 33], or in the carboxyl-terminal part, as for Pex11βp and Pex15p [24, 34]. Recent studies contradict these results of a single set of peroxisomal targeting information and suggest the presence of multiple targeting signals per membrane protein. They describe two distinct non-overlapping targeting regions in PMP47 of *Candida boidinii*, its human ortholog PMP34, in human Pex13p and in human PMP22 [35–37]. All mPTS sequences identified so far have two elements that are crucial for function. The first element is a basic cluster of about five amino acids [27, 32, 33, 36, 37]. Substituting two or more basic amino acids by alanines or glycines strongly decreases peroxisomal targeting efficiency [28, 32, 33, 36, 37]. The second element is the existence of one or more transmembrane domains within or close to the basic clusters [27, 30, 32, 36–40]. In Pex3p, Pex11βp, Pex14p and Pex22p, one single transmembrane domain is directly integrated in the targeting signal [24, 28, 30]. Two transmembrane domains are required for targeting of rat and human PMP22 [31, 37] and three for PMP34 [36].

A current model for the targeting and import of peroxisomal membrane proteins is summarized in figure 3. After synthesis in the cytosol, specific docking proteins pick up peroxisomal membrane proteins. Pex19p is a good candidate protein for such a chaperone-like docking or receptor protein [23, 41, 42] and positively charged basic clusters might mediate the binding. The membrane proteins complexed to their cytosolic docking protein are transported to the peroxisome membrane by specific targeting information. The complex associates with the peroxisome membrane either directly or by binding to a component of the peroxisomal membrane insertion machinery. The proteins are then integrated into the organelle membrane. The docking proteins shuttle back to the cytosol and the docking and insertion machinery can receive new substrates. This translocation apparatus might be even more complex and involve further cytosolic factors.

Targeting and import of matrix proteins

In contrast to peroxisome membrane formation, over the past years a clear picture has emerged as to how matrix proteins are targeted to peroxisomes. More than 95% of all known peroxisomal matrix proteins have a carboxyl-terminal peroxisomal targeting signal (PTS1) with the consensus sequence -S-K-L-COOH or a conservative variant [43–45]. A few matrix proteins have their peroxisomal targeting signal at the amino terminus (PTS2) with the consensus sequence (R/K)(L/I/V)X_5(H/Q)(L/A) [46]. The subsequent transport and import processes involve receptor and translocation complexes in the cytoplasm, on the peroxisome surface and in the peroxisome membrane. The current model for matrix protein import is illustrated in figure 4 [8, 47]. PTS1 and PTS2 containing peroxisomal matrix proteins are recognized by specific receptors in the cytosol. The PTS1 signal binds to the tetratricopeptide repeat (TPR) domains of the receptor protein Pex5p [48, 49] while the PTS2 signal interacts with the receptor protein Pex7p. After binding the corresponding ligands in the cytosol, the receptors and their cargo attach to components of the docking machinery in the peroxisomal membrane [19, 50, 51]. Transport through the cytosol is facilitated by several other peroxins. Pex1p and Pex6p are members of the AAA family of ATPases (ATPases associated with various cellular activ-

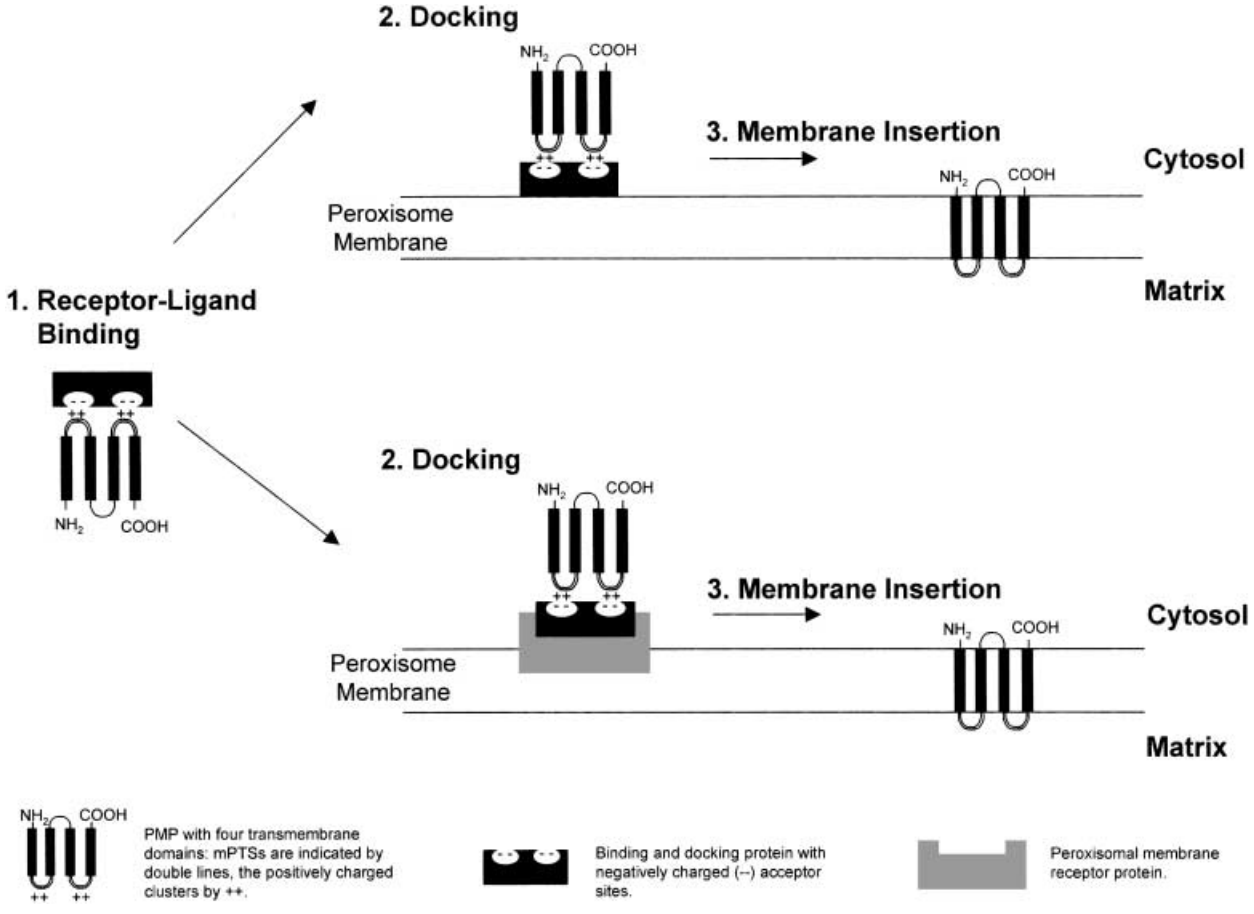


Figure 3. Hypothetical model for peroxisome membrane synthesis. After synthesis in the cytosol, peroxisomal membrane proteins are recognized and bound to a receptor. The receptor-ligand complex is transported to the surface of the peroxisome membrane. The peroxisomal membrane protein can be inserted directly, or the insertion requires additional receptor proteins in the peroxisome membrane.

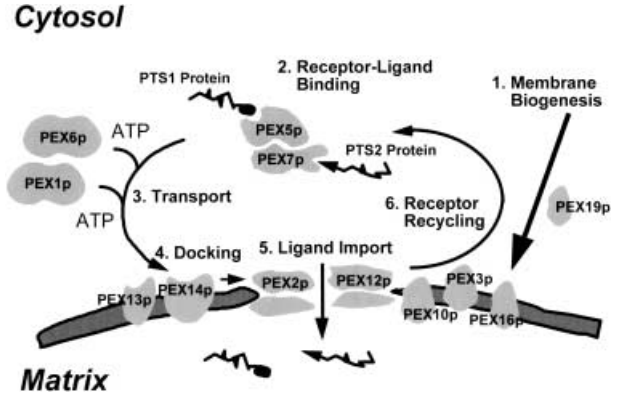


Figure 4. A hypothetical model for peroxisomal matrix protein import. Peroxisomal matrix proteins are synthesized in the cytosol. The import involves binding of newly synthesized proteins by cytosolic receptor proteins, transport of the receptor-ligand complex to the peroxisomal membrane, docking, matrix protein import, and receptor recycling.

ities) [52, 53]. They are required for the import of both PTS1 and PTS2 matrix proteins and seem to have a direct effect on receptor-ligand complex stability. At the peroxisomal membrane, the Pex5p and Pex7p-ligand complexes interact with Pex13p and Pex14p of the docking machinery. Pex13p is most likely responsible for anchoring Pex14p and binding Pex5 via its Src homology 3 (SH3) domain [54–56]. Pex14p, an integral peroxisomal membrane protein in *Hansenula polymorpha* [57] and humans [58] and a peroxisomal-membrane-associated protein in *Saccharomyces cerevisiae* [59], interacts with Pex5p, Pex7p and Pex13p [58–60]. In the following translocation step, receptor-ligand complexes are delivered to components of the translocation machinery and are translocated across the membrane. Components of the translocation machinery are Pex12p, Pex2p and Pex10p containing a C_3CHC_4 zinc-binding motif that binds two zinc atoms and is thought to mediate protein-protein interactions. After delivering their cargo, the Pex5p and Pex7p receptor proteins shuttle back to the cytosol and are able to receive new substrates for another cycle of protein import.

In humans, at least 12 peroxins are required for the targeting and import of peroxisomal matrix proteins. Defects in any of these peroxins will disrupt the protein import cycle and cause a peroxisomal biogenesis disorder.

Peroxisomal disorders

The importance of peroxisomes in humans is underlined by the existence of an expanding group of inborn errors of metabolism in which there is impairment of peroxisomal functions. The estimated incidence is 1:20,000–1:100,000 births [8]. They are subdivided into two major categories (table 2) [8, 15, 47, 61]. The first category is the single-protein defects in which a single metabolic function is deficient and peroxisome assembly is unaffected. Examples are X-linked adrenoleukodystrophy and autosomal recessive disorders like hyperoxaluria type I, Refsum disease and single β-oxidation enzyme defects. The second category is the peroxisome biogenesis disorders with deficiency of multiple peroxisome function. They form a genetically heterogeneous group of autosomal recessive disorders.

Table 2. Classification of peroxisomal disorders.

Single-protein defects
X-linked adrenoleukodystrophy
Acyl-CoA oxidase deficiency
Bifunctional protein deficiency
Thiolase deficiency
Acatalasemia
Refsum disease
Glutaryl-CoA oxidase deficiency (glutaric aciduria type 3)
Mevalonate kinase deficiency
Alanine glyoxylate aminotransferase deficiency (hyperoxaluria type I)
Peroxisome biogenesis disorders
Zellweger syndrome
Neonatal adrenoleukodystrophy
Infantile Refsum disease
Rhizomelic chondrodysplasia punctata
Hyperpipecolic acidemia

Peroxisome biogenesis disorders

Clinical phenotypes

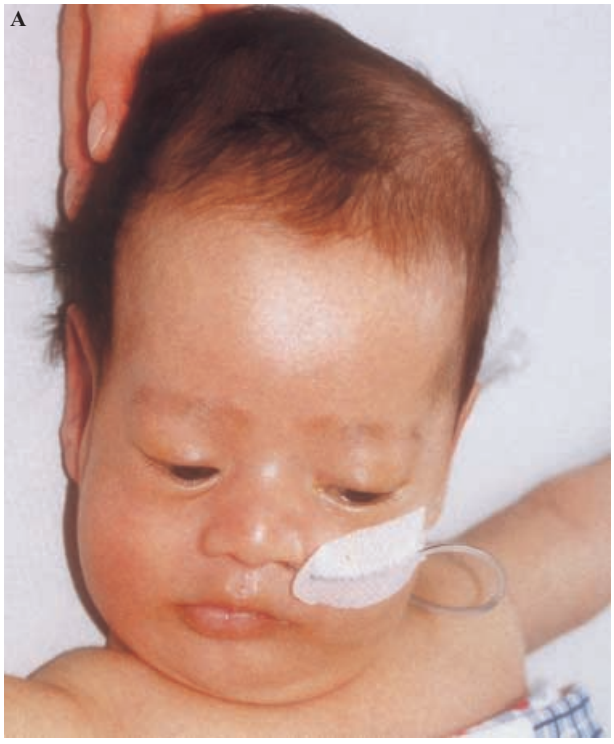
The clinical prototype of peroxisome biogenesis disorders is Zellweger syndrome, also referred to as cerebrohepatorenal syndrome. Diseases displaying similar but milder phenotypes than those of Zellweger syndrome patients are pseudo-Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum disease. Molecular studies have shown that these overlapping clinical entities have a common molecular background and are referred to as the Zellweger spectrum. A further but distinct phenotype of the group of peroxisome biogenesis disorders is rhizomelic chondrodysplasia punctata. The main clinical symptoms of peroxisome biogenesis disorders are summarized in table 3.

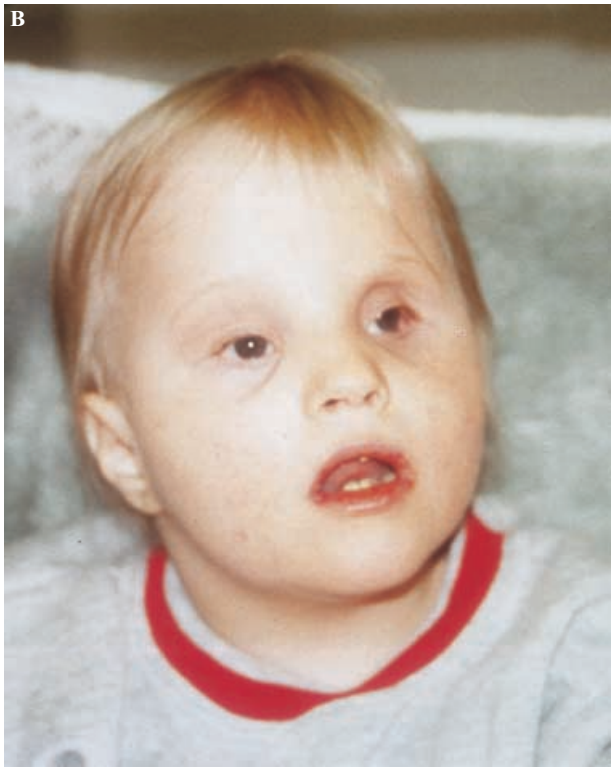
Zellweger syndrome is at the severe end of the Zellweger spectrum, while the others represent milder variants. Pa-

Table 3. Clinical symptoms of patients with peroxisome biogenesis disorders.

Abnormal features	Zellweger spectrum		Rhizomelic chondrodysplasia punctata
	severe form Zellweger syndrome	milder forms: pseudo-Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum disease	
Age of survival	< 1 year	≥ 1 year	< 2 year
Dysmorphic features			
Typical face	+++	++	++
Cerebral			
Hypotonia	+++	++	++
Epileptic seizures	++	++	++
Psychomotor retardation	+++	+++	+++
Gavage feeding	+++	++	++
Ocular			
Cataract	++	+	+++
Hepatorenal			
Liver fibrosis or cirrhosis	+++	++	++
Renal cysts	++	–	–
Skeletal system			
Calcific stippling	++	++	++
Rhizomelia	–	–	+++

+ mild; ++ moderate; +++ severe; – not present.





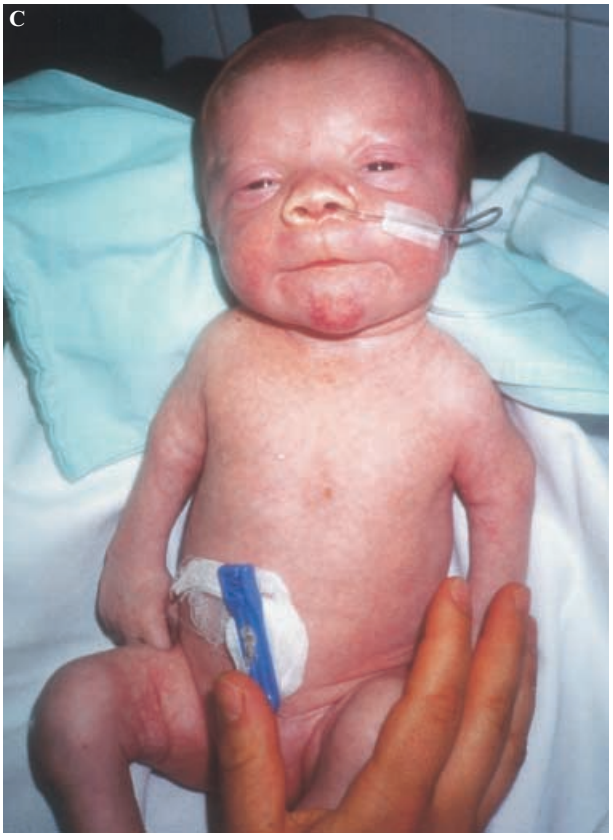


Figure 5. Typical facial appearance of patients with peroxisome biogenesis disorders. (*A*) Patient with Zellweger syndrome. (*B*) Zellweger spectrum patient with a milder clinical phenotype. (*C*) Patient with rhizomelic chondrodysplasia punctata; note also the shortening of proximal limbs.

tients with Zellweger syndrome have multiple congenital anomalies and rarely survive their first year of life. Affected infants have an abnormal facial appearance (fig. 5A) including a high forehead, a flat occiput (the bone forming the rear and rear base of the skull), a large fontanelle (the child's 'soft spot'), a broad nasal bridge, shallow orbital ridges (the ridge beneath the eyebrow) and a high arched palate [8]. They show severe hypotonia (poor muscle tone, or 'floppiness'), global developmental delay, feeding difficulty and seizures. Other typical features of the Zellweger spectrum are eye abnormalities, liver disease and renal cysts. Radiologic examination reveals abnormal calcifications ('calcific stippling') of patella and long bone epiphyses. Patients with milder variants of the Zellweger spectrum show similar though less pronounced clinical phenotypes and many patients survive several decades (fig. 5B). The typical clinical features for patients with rhizomelic chondrodysplasia punctata include abnormal facies, profound developmental delay, cataracts, abnormal calcifications of epiphyses and striking shortening of the proximal limbs ('rhizomelia') (fig. 5C). The majority of patients die in the first 2 years of life, but several are known to have survived longer.

Biochemical phenotypes

The biochemical phenotypes of peroxisome biogenesis disorders reflect the defect in organelle assembly. Cells from most Zellweger spectrum patients show numerous peroxisome-like structures that contain peroxisomal membrane proteins but are defective in the import of PTS1 and PTS2 matrix enzymes. These peroxisomal enzymes are mislocalized in the cytosol. They are unable to function properly and some of these enzymes degrade. Cells from a number of Zellweger spectrum patients lack peroxisomal membranes indicating a defect in the synthesis of the peroxisome membrane and concomitant mislocalization of PTS1 and PTS2 matrix enzymes. In contrast, cells from patients with rhizomelic chondrodysplasia punctata mislocalize only a small subset of peroxisomal matrix enzymes, namely PTS2 enzymes. In humans, these PTS2 enzymes are thiolase, phytanoyl-CoA hydroxylase and alkyl-dihydroxyacetonephosphate synthetase. The biochemical abnormalities of peroxisome biogenesis patients are summarized in table 4. In patients with Zellweger syndrome, the levels of very long chain fatty acids ($>C_{22}$), phytanic acid, pipecolic acid, pristanic acid and bile acid intermediates are increased; plasmalogen levels are reduced. Patients with rhizomelic chondrodysplasia punctata have normal levels of very long chain fatty acids but marked accumulation of phytanic acid due to a defect in the α-oxidation of this branched-chain fatty acid. They also have a profound defect in plasmalogen synthesis. Cells from Zellweger spectrum patients and those from patients with rhizomelic chondrodysplasia punctata can be distinguished by catalase immunofluorescence staining due to their differences in

Table 4. Biochemical abnormalities in patients with peroxisome biogenesis disorders.

Abnormal metabolite or function	Analysis material	Zellweger spectrum		Rhizomelic chondrodysplasia punctata
	severe form: Zellweger syndrome	milder forms: pseudo-Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum disease		
Very long chain fatty acids	plasma, fibroblasts, chorion cells, amniocytes	↑↑	↑	–
Phytanic acid	plasma, fibroblasts, chorion cells, amniocytes	↑	↑	↑↑
Pipecolic acid	plasma, fibroblasts, chorion cells, amniocytes	↑↑	↑	–
Bile acid intermediates	plasma, urine	↑↑	↑	–
Plasmalogen	erythrocytes	↓	↓	↓↓
Plasmalogen biosynthesis	fibroblasts, chorion cells, amniocytes	↓	↓	↓↓
Catalase import (PTS1 enzyme)	fibroblasts, chorion cells, amniocytes	↓↓	↓↓	–
Thiolase processing	fibroblasts, chorion cells, amniocytes	↓	↓ or –	↓
Thiolase import (PTS2 enzyme)	fibroblasts, chorion cells, amniocytes	↓ or –	↓ or –	↓

↓ decreased, ↓↓ strongly decreased, ↑ increased, ↑↑ strongly increased, – not affected. PTS, peroxisomal targeting signal.

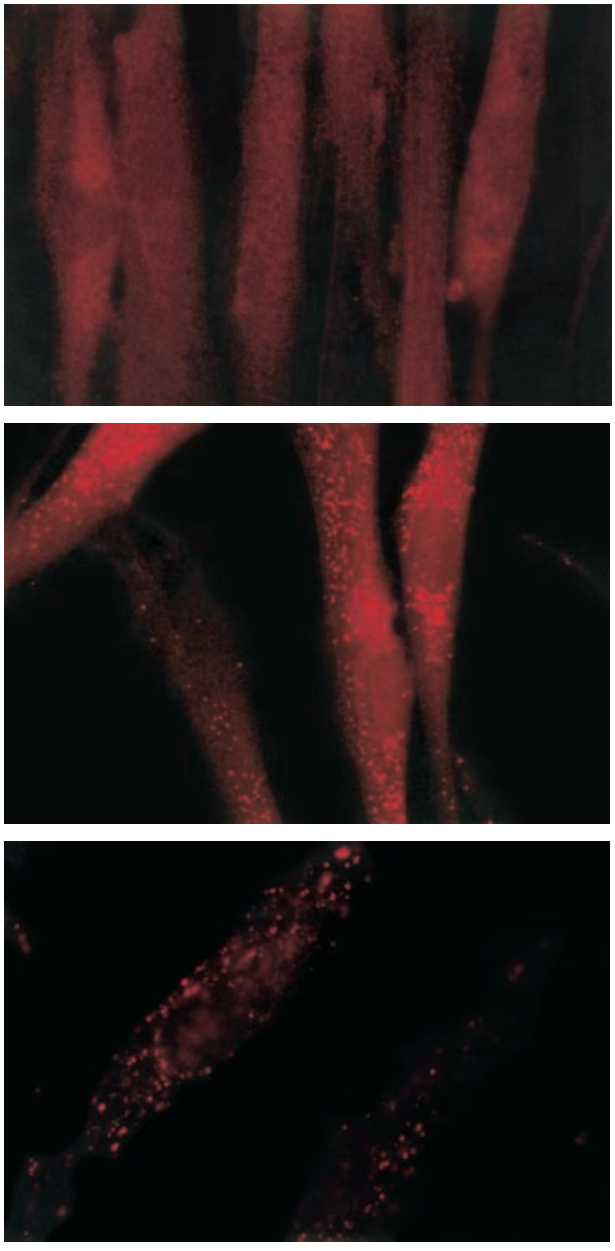


Figure 6. Subcellular localization of catalase by immunofluorescence staining (original magnification × 850). (*A*) Skin fibroblasts from a patient with Zellweger syndrome. The cells are unable to import catalase into peroxisome-like structures. The enzyme is distributed in the cytoplasm resulting in a diffuse staining pattern. (*B*) Skin fibroblasts from a patient with rhizomelic chondrodysplasia punctata. The enzyme is imported into the peroxisome resulting in a punctuate staining pattern. (*C*) Skin fibroblasts from an unaffected individual. The enzyme is localized in the peroxisomal matrix resulting in a typical punctuate staining pattern.

importing PTS1 and PTS2 enzymes. Zellweger spectrum cells show a homogeneous distribution of catalase in the cytoplasm (fig. 6A). In contrast, normal cells and rhizomelic chondrodysplasia punctata cells reveal a punctuate staining pattern reflecting conserved PTS1 protein import and the accumulation of catalase in the peroxisomal matrix (fig. 6B, C).

Molecular aspects

Peroxisome biogenesis disorders are inherited in an autosomal recessive manner. They are caused by mutations in *PEX* genes that encode peroxins, proteins involved in peroxisome assembly and proliferation. So far, somatic cell fusion studies have found 12 complementation groups indicating that at least 12 different genes contribute to human peroxisome biogenesis [8, 15]. Different experimental approaches including functional complementation, functional cloning and homology probing have been successful in identifying the primary genetic defect in 11 of the 12 known complementation groups other than complementation group 8 (table 5). Functional complementation of mutant Chinese hamster ovary cell lines with rat and human cDNA expression libraries led to the first identification of a gene related to human peroxisome biogenesis disorder, *PEX2,* which is defective in CG10 [62–64]. This technique was also used to identify the genes defective in complementation group 1, *PEX1* [65], complementation group 3, *PEX12* [66, 67], complementation group 4, *PEX6* [68], and complementation group 14, *PEX19* [23]. This gene identification strategy used homology probing of mammalian databases with known yeast sequences. Numerous peroxisome biogenesis factors had been characterized in lower eukaryotes including *S. cerevisiae* [18, 19, 69], *P. pastoris* [70, 71], *Y. lipolytica* [72] and *H. polymorpha* [39, 73]. The yeast peroxin sequences were used for a computer-based screen of human expressed-sequence tag databases. This second approach identified the *PEX1*, *PEX3*, *PEX5*, *PEX6*, *PEX7*, *PEX10*, *PEX12*, *PEX13* and *PEX16* genes that are defective in complementation group 1 [74], 12 [22, 38], 2 [49], 4 [75], 11 [76–78], 7 [79], 3 [80], 13 [81] and 9 [82], respectively. Zellweger spectrum patients are distributed among 11 complementation groups and their defects result from mutations in different *PEX* genes. In contrast, patients with rhizomelic chondrodysplasia punctata are restricted to a single group, complementation group 11, and their defects result from mutations in the *PEX7* gene. Most complementation groups include only a few patients. An exception to this general rule is complementation group 1, by far the largest complementation group, including more than half of all peroxisome biogenesis defect patients [83–86]. The group results from mutations in the *PEX1* gene, which encodes an AAA protein of the family of ATPases associated with various cellular activities [53, 74]. A variety of *PEX1* mutant alleles have been described [74, 83–87]. Of these, the insertion c.2097-2098insT and a single missense mutation c.2528 G > A, resulting in G843D, account for more than half of all *PEX1* mutations. The mutant proteins encoded by various

Table 5. Human PEX genes and peroxisome biogenesis disorders.

Gene	Complementation group [83]	Clinical phenotype	Peroxin motifs and functions	References
PEX1	CG1	ZS, NALD, IRD	AAA ATPase, matrix protein import	74, 84, 93
PEX2	CG10	ZS, IRD	zinc RING, matrix protein import	63, 94, 95, 96
PEX3	CG12	ZS	no known motif, peroxisome membrane synthesis	68, 97
PEX5	CG2	ZS, NALD, IRD	TPR domain, matrix protein import, PTS1 receptor	49, 98
PEX6	CG4	ZS, NALD	AAA ATPase, matrix protein import	8, 93
PEX7	CG11	RCDP	WD-40, matrix protein import, PTS2 receptor	76, 77, 78, 99
PEX10	CG7	ZS, NALD	zinc RING, matrix protein import	79, 100, 101
PEX11	?	–	no known motif, peroxisome proliferation	102, 103
PEX12	CG3	ZS, NALD, IRD	zinc RING, matrix protein import	80, 101
PEX13	CG13	ZS, NALD	SH3 domain, matrix protein import, receptor docking	54
PEX14	?	–	no known motif, matrix protein import, receptor docking	59
PEX16	CG9	ZS	no known motif, peroxisome membrane synthesis	82
PEX19	CG14	ZS	farnesylation, peroxisome membrane synthesis, putative PMP receptor	24
?	CG8	ZS, NALD, IRD	?, matrix protein import	8

CG, complementation group; ZS, Zellweger syndrome; NALD, neonatal adrenoleukodystrophy; IRD, infantile Refsum disease; RCDP, rhizomelic chondrodysplasia punctata; AAA ATPase, ATPases associated with various cellular activities; PMP, peroxisomal membrane protein; PTS1, peroxisomal targeting signal 1; PTS2, peroxisomal targeting signal 2; SH3, Src homology 3; TPR, tetratricopeptide repeat; WD-40, repeat containing approximately 40 amino acids with a central Trp-Asp motif.

PEX1 alleles explain, in part, the heterogeneous clinical phenotypes of Zellweger spectrum patients. A complete lack of Pex1 protein was found to be associated with severe phenotypes, while residual amounts of Pex1 protein lead to milder forms of disease. The c.2097-2098insT leads to a premature termination codon within the AAA domain and a total loss of Pex1 protein activity [88]. In contrast, the mutation c.2528 G > A encodes a Pex1 mutant protein with residual import activity for peroxisomal matrix proteins [74, 84, 89]. The mutation seems to result in a misfolded protein, which is more stable at lower than at higher temperatures. The G843D mutation most likely disrupts the protein-protein interaction between Pex1p and Pex6p. *PEX6* encodes another peroxin of the AAA ATPase family and is the gene defective in complementation group 4 [52, 90].

Although the pathogenesis of peroxisome biogenesis disorders is poorly understood, there seems to be a general correlation between disease severity and peroxin functions. Similarly to complementation group 1, Zellweger spectrum patients in complementation group 3 with a residually active Pex12 protein have a less severe clinical phenotype than those with undetectable protein [91]. Another example in patients with rhizomelic chondrodysplasia punctata is L292ter, which accounts for more than half of all *PEX7* mutations and produces a mutant Pex7 protein with no residual activity [76, 78]. Patients homozygous for L292ter have severe biochemical defects and exhibit a severe clinical phenotype. In contrast, mutant *PEX7* alleles encoding a protein with residual activity are found in patients with milder forms of disease [92].

Eight of the 11 PEX genes known to be defective in patients with peroxisome biogenesis disorders affect peroxisomal matrix protein import. Although the gene for complementation group 8 is not known, biochemical studies in patient cell lines suggest a role in peroxisomal matrix protein import. In yeast, *PEX14* also participates in peroxisomal matrix protein import and its human homolog might have a similar role. Defects in peroxisome membrane synthesis have been found in only three complementation groups of Zellweger spectrum patients with mutations in any of three genes, *PEX3*, *PEX16* and *PEX*19. There are other anticipated *PEX* genes defective in additional and as yet unknown complementation groups of peroxisome biogenesis disorders. In yeast and Chinese hamster ovary cell lines, more than 12 peroxins are required for peroxisomal membrane synthesis or matrix protein import. The human homologs of these additional *PEX* genes represent excellent candidates for identifying additional genes defective in peroxisome biogenesis disorders in the future.

Conclusions and further directions

The interactive application of biochemistry, cell biology, clinical analysis, pathology and genetics in recent years have advanced our understanding of the role of *PEX* genes in peroxisome assembly and the pathogenesis of peroxisome biogenesis disorders. Eleven human *PEX* genes defective in these disorders have been identified and numerous mutations described. Functional analyses of the encoded peroxins have elucidated a phenotype-

genotype correlation. Rudimentary hypothetical models for most complicated biological processes, the synthesis of peroxisome membranes and the import of peroxisomal matrix proteins have been developed. The increasing activity in the peroxisome research field promises a comprehensive understanding of the molecular mechanisms of peroxisome biogenesis in the near future. These advances will hopefully promote attempts at devising effective therapies, especially for those patients with milder phenotypes.

1 Dansen T. B., Wirtz K. W., Wanders R. J. and Pap E. H. (2000) Peroxisomes in human fibroblasts have a basic pH. Nat. Cell Biol. **2:** 51–53
2 Lazarow P. B. and Fujiki Y. (1985) Biogenesis of peroxisomes. Annu. Rev. Cell Biol. **1:** 489–530
3 Fahimi H. D., Reich D., Volkl A. and Baumgart E. (1996) Contributions of the immunogold technique to investigation of the biology of peroxisomes. Histochem. Cell Biol. **106:** 105–114
4 Espeel M., Depreter M., Nardacci R., D'Herde K., Kerckaert I., Stefanini S. et al. (1997) Biogenesis of peroxisomes in fetal liver. Microsc. Res. Tech. **39:** 453–466
5 Chang C. C., South S., Warren D., Jones J., Moser A. B., Moser H. W. et al. (1999) Metabolic control of peroxisome abundance. J. Cell Sci. **112:** 1579–1590
6 Fujiki Y., Rachubinski R. A. and Lazarow P. B. (1984) Synthesis of a major integral membrane polypeptide of rat liver peroxisomes on free polysomes. Proc. Natl. Acad. Sci. USA **81:** 7127–7131
7 Suzuki Y., Orii T., Takiguchi M., Mori M., Hijikata M. and Hashimoto T. (1987) Biosynthesis of membrane polypeptides of rat liver peroxisomes. J. Biochem. **101:** 491–496
8 Gould S. J., Raymond G. V. and Valle D. (2001) The peroxisome biogenesis disorders. In: The Metabolic and Molecular Bases of Inherited Disease, pp. 3181–3217, Scriver C. R., Beaudet A. L., Valle D. and Sly W. S. (eds), McGraw-Hill, New York
9 Titorenko V. I. and Rachubinski R. A. (2001) The life cycle of the peroxisome. Nat. Rev. Mol. Cell Biol. **2:** 357–368
10 Distel B., Erdmann R., Gould S. J., Blobel G., Crane D. I., Cregg J. M. et al. (1996) Mini-review: a unified nomenclature for peroxisome biogenesis factors. J. Cell Biol. **135:** 1–3
11 Herrmann J. M., Malkus P. and Schekman R. (1999) Out of the ER – outfitters, escorts and guides. Trends Cell Biol. **9:** 5–7
12 Neupert W. (1997) Protein import into mitochondria. Annu. Rev. Biochem. **66:** 863–917
13 Nickel W. and Wieland F. T. (1998) Biosynthetic protein transport through the early secretory pathway. Histochem. Cell Biol. **109:** 477–486
14 Kuehn M. J. and Schekman R. (1997) COPII and secretory cargo capture into transport vesicles. Curr. Opin. Cell Biol. **9:** 477–483
15 Gould S. J. and Valle D. (2000) Peroxisome biogenesis disorders: genetics and cell biology. Trends Genet. **16:** 340–345
16 Titorenko V. I. and Rachubinski R. A. (1998) The endoplasmic reticulum plays an essential role in peroxisome biogenesis. Trends Biochem. Sci. **23:** 231–233
17 Kunau W. H. and Erdmann R. (1998) Peroxisome biogenesis: back to the endoplasmic reticulum? Curr. Biol. **8:** R299–R302
18 Kunau W. H. (1998) Peroxisome biogenesis: from yeast to man. Curr. Opin. Microbiol. **1:** 232–237
19 Tabak H. F., Braakman I. and Distel B. (1999) Peroxisomes: simple in function but complex in maintenance. Trends Cell Biol. **9:** 447–453
20 Waterham H. R., Titorenko V. I., Swaving G. J., Harder W. and Veenhuis M. (1993) Peroxisomes in the methylotrophic yeast *Hansenula polymorpha* do not necessarily derive from pre-existing organelles. EMBO J. **12:** 4785–4794
21 South S. and Gould S. J. (1999) Peroxisome synthesis in the absence of pre-existing peroxisomes. J. Cell Biol. **144:** 255–266
22 South S. T., Sacksteder K. A., Li X., Liu Y. and Gould S. J. (2000) Inhibitors of COPI and COPII do not block *PEX3*-mediated peroxisome synthesis. J. Cell Biol. **149:** 1345–1360
23 Matsuzono Y., Kinoshita N., Tamura S., Shimozawa N., Hamasaki M., Ghaedi K. et al. (1999) Human *PEX19*: cDNA cloning by functional complementation, mutation analysis in a patient with Zellweger syndrome, and potential role in peroxisomal membrane assembly. Proc. Natl. Acad. Sci. USA **96:** 2116–2121
24 Sacksteder K. A., Jones J. M., South S. T., Li X., Liu Y. and Gould S. J. (2000) Pex19 binds multiple peroxisomal membrane proteins, is predominantly cytoplasmic, and is required for peroxisome membrane synthesis. J. Cell Biol. **148:** 931–944
25 Novikoff A. B. and Shin W. Y. (1964) The endoplasmic reticulum in the golgi zone and its relations to microbodies, golgi apparatus and autophagic vacuoles in rat liver cells. J. Micros. **3:** 187–206
26 Titorenko V. I. and Rachubinski R. A. (1998) Mutants of the yeast *Yarrowia lipolytica* defective in protein export exit from the endoplasmic reticulum are also defective in peroxisome biogenesis. Mol. Cell Biol. **18:** 2789–2803
27 Biermanns M. and Gärtner J. (2001) Targeting elements in the amino-terminal part direct the human 70 kDa peroxisomal integral membrane protein (PMP70) to peroxisomes. Biochem. Biophys. Res. Commun. **285:** 649–655
28 Koller A., Snyder W. B., Faber N. K., Wenzel T. J., Rangell L., Keller G. A. et al. (1999) Pex22p of *Pichia pastoris*, essential for peroxisomal matrix protein import, anchors the ubiquitin-conjugating enzyme, Pex4p, on the peroxisomal membrane. J. Cell Biol. **146:** 99–112
29 Kammerer S., Holzinger A., Welsch U. and Roscher A. A. (1998) Cloning and characterization of the gene encoding the human peroxisomal assembly protein Pex3p. FEBS **429:** 53–60
30 Soukupova M., Sprenger C., Gorgas K., Kunau W. H. and Dodt G. (1999) Identification and characterization of the human peroxin Pex3. Eur. J. Cell Biol. **78:** 357–374
31 Pause B., Saffrich R., Hunziker A., Ansorge W. and Just W. W. (2000) Targeting of the 22 kDa integral peroxisomal membrane protein. FEBS Letters **471:** 23–28
32 Dyer J. M., McNew J. A. and Goodman J. M. (1996) The sorting sequence of the peroxisomal integral membrane protein PMP47 is contained within a short hydrophilic loop. J. Cell Biol. **133:** 269–280
33 Honsho M. and Fujiki Y. (2001) Topogenesis of peroxisomal membrane protein requires a short, positively charged intervening-loop sequence and flanking hydrophopic segments. J. Biol. Chem. **276:** 9375–9382
34 Elgersma Y., Kwast L., Berg M. van den, Snyder W. B., Distel B., Subramani S. et al. (1997) Overexpression of Pex15p, a phosphorylated peroxisomal integral membrane protein required for peroxisome assembly in *S. cerevisiae*, causes proliferation of the endoplasmic reticulum membrane. EMBO J. **16:** 7326–7341
35 Jones M. J., Morrell J. C. and Gould S. J. (2001) Multiple distinct targeting signals in integral peroxisomal membrane proteins. J. Cell Biol. **153:** 1141–1149
36 Wang X., Unruh M. J. and Goodman J. M. (2001) Discrete targeting signals direct Pmp47 to oleate-induced peroxisomes in *Saccharomyces cerevisiae*. J. Biol. Chem. **276:** 10897–10905

37 Brosius U., Dehmel T. and Gärtner J. (2002) Two different targeting signals direct human peroxisomal membrane protein 22 to peroxisomes. J. Biol. Chem. **277:** 774–784

38 Höhfeld J., Veenhuis M. and Kunau W. H. (1991) PAS3, a *Saccharomyces cerevisiae* gene encoding a peroxisomal integral membrane protein essential for peroxisomal biogenesis. J. Cell Biol. **114:** 1167–1175

39 Baerends R. J. S., Rasmussen S. W., Hilbrands R. E., Heide M. van der, Faber K. N., Reuvekamp P. T. W. et al. (1996) The *Hansenula polymorpha PEX9* gene encodes a peroxisomal membrane protein essential for peroxisome assembly and integrity. J. Biol. Chem. **271:** 8887–8894

40 Wiemer E. A. C., Luers G., Faber K. N., Wenzel T., Veenhuis M. and Subramani S. (1996) Isolation and characterization of Pas2p, a peroxisomal membrane protein essential for peroxisome biogenesis in the methylotrophic yeast *Pichia pastoris*. J. Biol. Chem. **271:** 18973–18980

41 Götte K., Girzalsky W., Linkert M., Baumgart E., Kammerer S., Kunau W. H. et al. (1998) Pex19p, a farnesylated protein essential for peroxisome biogenesis. Mol. Cell Biol. **18:** 616–628

42 Snyder W. B., Koller A., Choy A. J. and Subramani S. (2000) The peroxin Pex19p interacts with multiple, integral membrane proteins at the peroxisomal membrane. J. Cell Biol. **149:** 1171–1177

43 Gould S. J., Keller G. A. and Subramani S. (1988) Identification of peroxisomal targeting signals located at the carboxy terminus of four peroxisomal proteins. J. Cell Biol. **107:** 897–905

44 Gould S. J., Keller G. A., Hosken N., Wilkinson J. and Subramani S. (1989) A conserved tripeptide sorts proteins to peroxisomes. J. Cell Biol. **108:** 1657–1664

45 Subramani S. (1993) Protein import into peroxisomes and biogenesis of the organelle. Annu. Rev. Cell Biol. **9:** 445–454

46 Swinkels B. W., Gould S. J., Bodnar A. G., Rachubinski R. A. and Subramani S. (1991) A novel, cleavable peroxisomal targeting signal at the rat 3-ketoacyl-CoA thiolase. EMBO J. **10:** 3255–3262

47 Gärtner J. (2000) Disorders related to peroxisomal membranes. J. Inherit. Metab. Dis. **23:** 264–272

48 Brocard C., Kragler F., Simon M. M., Schuster T. and Hartig A. (1994) The tetratricopeptide repeat-domain of the PAS10 protein of *Saccharomyces cerevisiae* is essential for binding the peroxisomal targeting signal -SKL. Biochem. Biophys. Res. Commun. **204:** 1016–1022

49 Dodt G., Braverman N., Wong C., Moser A., Moser H. W., Watkins P. et al. (1995) Mutations in the PTS1 receptor gene, PXR1, define complementation group 2 of the peroxisome biogenesis disorders. Nat. Genet. **9:** 115–125

50 Dodt G. and Gould S. J. (1996) Multiple *PEX* genes are required for proper subcellular distribution and stability of Pex5p, the PTS1 receptor: evidence that PTS1 protein import is mediated by a cycling receptor. J. Cell Biol. **135:** 1763–1774

51 Braverman N., Dodt G., Gould S. J. and Valle D. (1995) Disorders of peroxisome biogenesis. Hum. Mol. Genet. **4:** 1791–1798

52 Geisbrecht B. V., Collins C. S., Reuber B. E. and Gould S. J. (1998) Disruption of a Pex1-Pex6 interaction is the most common cause of the neurologic disorders Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile refsum disease. Proc. Natl. Acad. Sci. USA **95:** 8630–8635

53 Faber K. N., Heyman J. A. and Subramani S. (1998) Two AAA family peroxins, PpPex1p and PpPex6p, interact with each other in an ADP-dependent manner and are associated with different subcellular membranous structures distinct from peroxisomes. Mol. Cell. Biol. **18:** 936–943

54 Gould S. J., Kalish J. E., Morrell J. C., Bjorkman J., Urquhart A. J. and Crane D. I. (1996) Pex13p is an SH3 protein of the peroxisome membrane and a docking factor for the predominantly cytoplasmic PTS1 receptor. J. Cell Biol. **135:** 85–95

55 Urquhart A. J., Kennedy D., Gould S. J. and Crane D. I. (2000) Interaction of pex5p, the type 1 peroxisome targeting signal receptor, with the peroxisomal membrane proteins pex14p and pex13p. J. Biol. Chem. **275:** 4127–4136

56 Bottger G., Barnett P., Klein A. T., Kragt A., Tabak H. F. and Distel B. (2000) *Saccharomyces cerevisiae* PTS1 receptor Pex5p interacts with the SH3 domain of the peroxisomal membrane protein pex13p in an unconventional, non-PXXP-related manner. Mol. Biol. Cell **11:** 3963–3976

57 Komori M., Kiel J. A. and Veenhuis M. (1999) The peroxisomal membrane protein Pex14p of *Hansenula polymorpha* is phosphorylated in vivo. FEBS Lett. **457:** 397–399

58 Will G. K., Soukupova M., Hong X., Erdmann K. S., Kiel J. A. K. W., Dodt G. et al. (1999) Identification and characterization of the human orthologue of yeast Pex14p. Mol. Cell Biol. **19:** 2265–2277

59 Albertini M., Rehling P., Erdmann R., Girzalsky W., Kiel J. A. K. W., Veenhuis M. et al. (1997) Pex14p, a peroxisomal membrane protein binding both receptors of the two PTS-dependent import pathways. Cell **89:** 83–92

60 Brocard C., Lametschwandner G., Koudelka R. and Hartig A. (1997) Pex14p is a member of the protein linkage map of Pex5p. EMBO J. **16:** 5491–5500

61 Moser H. W., Smith K. D., Watkins, P. A., Powers J. and Moser A. B. (2001) X-linked adrenoleukodystrophy. In: The Metabolic and Molecular Bases of Inherited Disease, pp. 3257–3301, Scriver C. R., Beaudet A. L., Valle D. and Sly W. S. (eds), McGraw-Hill, New York

62 Tsukamoto T., Yokota S. and Fujiki Y. (1990) Isolation and characterization of Chinese hamster ovary cell mutants defective in assembly of peroxisomes. J. Cell Biol. **110:** 651–660

63 Tsukamoto T., Miura S. and Fujiki Y. (1991) Restoration by a 35 K membrane protein of peroxisome assembly in a peroxisome-deficient mammalian cell mutant. Nature **350:** 77–81

64 Shimozawa N., Tsukamoto T., Suzuki Y., Orii T., Shirayoshi Y., Mori T. et al. (1991) A human gene responsible for Zellweger syndrome that affects peroxisome assembly. Science **255:** 1132–1134

65 Tamura S., Okumoto K., Toyama R., Shimozawa N., Tsukamoto T., Suzuki Y. et al. (1998) Human *PEX1* cloned by functional complementation on a CHO cell mutant is responsible for peroxisome-deficient Zellweger syndrome of complementation group I. Proc. Natl. Acad. Sci. USA **95:** 4350–4355

66 Okumoto K. and Fujiki Y. (1997) *PEX12* encodes an integral membrane protein of peroxisomes. Nat. Genet. **17:** 265–266

67 Okumoto K., Shimozawa N., Kawai A., Tamura S., Tsukamoto T., Osumi T. et al. (1998) *PEX12*, the pathogenic gene of group III Zellweger syndrome: cDNA cloning by functional complementation on a CHO cell mutant, patient analysis, and characterization of Pex12p. Mol. Cell Biol. **18:** 4324–4336

68 Fukuda S., Shimozawa N., Suzuki Y., Zhang Z., Tomatsu S., Tsukamoto T. et al. (1996) Human peroxisome assembly factor-2 (PAF-2): a gene responsible for group C peroxisome biogenesis disorder in humans. Am. J. Hum. Genet. **59:** 1210–1220

69 Erdmann R., Veenhuis M., Mertens D. and Kunau W. H. (1989) Isolation of peroxisome-deficient mutants of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **86:** 2432–2436

70 Spong A. P. and Subramani S. (1993) Cloning and characterization of PAS5: a gene required for peroxisome biogenesis in the methylotrophic yeast *Pichia pastoris*. J. Cell Biol. **123:** 535–548

71 Erdmann R., Veenhuis M. and Kunau W. H. (1997) Peroxisome biogenesis at the cross-roads. Trends Cell Biol. **7:** 400–407

72 Nuttley W. M., Brade A. M., Eitzen G. A., Veenhuis M., Aitchison J. D., Szilard R. K. et al. (1994) PAS4, a gene required for peroxisome assembly in the yeast *Yarrowia lipolytica*, encodes a novel member of a family of putative ATPases. J. Biol. Chem. **269:** 556–566

73 Klei I. J. van der, Hilbrands R. E., Swaving G. J., Waterham H. R., Vrieling E. G., Titorenko V. I. et al. (1995) The *Hansenula polymorpha PER3* gene is essential for the import of PTS1 proteins into the peroxisomal matrix. J. Biol. Chem. **270:** 17229–17236

74 Portsteffen H., Beyer A., Becker E., Epplen C., Pawlak A., Kunau W. H. et al. (1997) Human *PEX1* is mutated in complementation group 1 of the peroxisome biogenesis disorders. Nat. Genet. **17:** 449–452

75 Yahraus T., Braverman N., Dodt G., Kalish J. E., Morrell J. C., Moser H. W. et al. (1996) The peroxisome biogenesis disorder group 4 gene, PXAAA1, encodes a cytoplasmic ATPase required for stability of the PTS1 receptor. EMBO J. **15:** 2914–2923

76 Braverman N., Steel G., Obie C., Moser A., Moser H., Gould S. J. et al. (1997) Human *PEX7* encodes the peroxisomal PTS2 receptor and is responsible for rhizomelic chondrodysplasia punctata. Nat. Genet. **15:** 369–376

77 Purdue P. E., Zhang J. W., Skoneczny M. and Lazarow P. B. (1996) Rhizomelic chondrodysplasia punctata is caused by deficiency of human *PEX7*, a homologue of the yeast PTS2 receptor. Nat. Genet. **15:** 381–384

78 Motley A. M., Hettema E. H., Hogenhout E. M., Brites P., Asbroek A. L. M. A. ten, Wijburg F. A. et al. (1997) Rhizomelic chondrodysplasia punctata is a peroxisomal protein targeting disease caused by a non-functional PTS2 receptor. Nat. Genet. **15:** 377–380

79 Warren D. S., Morrell J. C., Moser H. W., Valle D. and Gould S. J. (1998) Identification of *PEX10*, the gene defective in complementation group 7 of the peroxisome biogenesis disorders. Am. J. Hum. Genet. **63:** 347–359

80 Chang C. C., Lee W. H., Moser H., Valle D. and Gould S. J. (1997) Isolation of the human *PEX12* gene, mutated in group 3 of the peroxisome biogenesis disorder patients. Nat. Genet. **15:** 385–388

81 Liu Y., Björkman J., Urquhart A., Wanders J. R. A., Crane D. I. and Gould S. J. (1999) *PEX13* is mutated in complementation group 13 of peroxisome-biogenesis disorders. Am. J. Hum. Genet. **65:** 621–634

82 Honsho M., Tamura S., Shimozawa N., Suzuki Y., Kondo N. and Fujiki Y. (1998) Mutation in *PEX16* is causal in the peroxisome-deficient Zellweger syndrome of complementation group D. Am. J. Hum. Genet. **63:** 1622–1630

83 Moser A. B., Rasmussen M., Naidu S., Watkins P. A., McGuinness M., Hajra A. K. et al. (1995) Phenotype of patients with peroxisomal disorders subdivided into sixteen complementation groups. J. Pediatr. **127:** 13–22

84 Reuber B. E., Germain-Lee E., Collins C. S., Morrell J. C., Ameritunga R., Moser H. W. et al. (1997) Mutations in *PEX1* are the most common cause of peroxisome biogenesis disorders. Nat. Genet. **17:** 445–448

85 Maxwell M. A., Nelson P. V., Chin S. J., Paton B. C., Carey W. F. and Crane D. I. (1999) A common *PEX1* frameshift mutation in patients with disorders of peroxisome biogenesis correlates with the severe Zellweger syndrome phenotype. Hum. Genet. **105:** 38–44

86 Wanders R. J. A., Mooijer P. A., Dekker C., Suzuki Y. and Shimozawa N. (1999) Disorders of peroxisome biogenesis: complementation analysis shows genetic heterogeneity with strong overrepresentation of one group (*PEX1* deficiency). J. Inherit. Metab. Dis. **22:** 314–318

87 Gärtner J., Preuss N., Brosius U. and Biermanns M. (1999) Mutations in *PEX1* in peroxisome biogenesis disorders: G843D and a mild clinical phenotype. J. Inherit. Metab. Dis. **22:** 311–313

88 Collins C. S. and Gould S. J. (1999) Identification of a common *PEX1* mutation in Zellweger syndrome. Hum. Mutat. **14:** 45–53

89 Walter C., Gootjes J., Mooijer P. A., Portsteffen H., Klein C., Waterham H. R. et al. (2001) Disorders of peroxisome biogenesis due to mutations in *PEX1*: phenotypes and Pex1 protein levels. Am. J. Hum. Genet. **69:** 35–48

90 Tamura S., Shimozawa N., Suzuki Y., Tsukamoto T., Osumi T. and Fujiki Y. (1998) A cytoplasmic AAA family peroxin, Pex1p, interacts with Pex6p. Biochem. Biophys. Res. Commun. **245:** 883–886

91 Chang C. C. and Gould S. J. (1998) Phenotype-genotype relationships in complementation group 3 of the peroxisome-biogenesis disorders. Am. J. Hum. Genet. **63:** 1294–1306

92 Braverman N., Lin P., Steel G., Obie C., Moser H., Moser A. et al. (1999) Mutation analysis of *PEX7* in patients with rhizomelic chondrodysplasia punctata. Am. J. Hum. Genet. **65:** A286

93 Collins C. S., Kalish J. E., Morrell J. C. and Gould S. J. (2000). The peroxisome biogenesis factors Pex4p, Pex22p, Pex1p, and Pex6p act in the terminal steps of peroxisomal matrix protein import. Mol. Cell. Biol. **20:** 7516–7526

94 Lovering R., Hanson I. M., Borden K. L. B., Martin S., O´Reilly N. J., Evan G. I. et al. (1993) Identification and preliminary characterization of a protein motif related to the zinc finger. Proc. Natl. Acad. Sci. USA **90:** 2112–2116

95 Patarca R. and Fletcher M. A. (1992) Ring finger in the peroxisome assembly factor-1. FEBS Lett. **312:** 1–2

96 Shimozawa N., Suzuki Y., Orii T., Moser A., Moser H. W. and Wanders R. J. A. (1993) Standardization of complementation grouping of peroxisome-deficient disorders and the second Zellweger patient with peroxisomal assembly factor-1 (PAF-1) defect. Am. J. Hum. Genet. **52:** 843–844

97 Ghaedi K., Honsho M., Shimozawa N., Suzuki Y., Kondo N. and Fujiki Y. (2000) *PEX3* is the causal gene responsible for peroxisomal membrane assembly-defective Zellweger syndrome of complementation group G. Am. J. Hum. Genet. **67:** 976–981

98 Leij I. van der, Franse M. M., Elgersma Y., Distel B. and Tabak H. F. (1993) PAS10 is a tetratricopeptide-repeat protein that is essential for the import of most matrix proteins into peroxisomes of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci USA **90:** 11782–11786

99 Marzioch M., Erdmann R., Veenhuis M. and Kunau W. H. (1994) PAS7 encodes a novel yeast member of the WD-40 protein family essential for import of 3-oxoacyl-CoA thiolase, a PTS2-containing protein, into peroxisomes. EMBO J. **13:** 4908–4918

100 Okumoto K., Itoh R., Shimozawa N., Suzuki Y., Tamura S., Kondo N. et al. (1998) Mutations in *PEX10* are the cause of Zellweger peroxisome deficiency syndrome of complementation group B. Hum. Mol. Genet. **7:** 1399–1405

101 Okumoto K., Abe I. and Fujiki Y. (2000) Molecular anatomy of the peroxin Pex12p: ring finger domain is essential for Pex12p function and interacts with the peroxisome-targeting signal type 1-receptor Pex5p and a ring peroxin, Pex10p. J. Biol. Chem. **275:** 25700–25710

102 Marshall P. A., Krimkevich Y. I., Lark R. H., Dyer J. M., Veenhuis M. and Goodman J. M. (1995) PMP27 promotes peroxisome proliferation. J. Cell Biol. **129:** 345–355

103 Abe I., Okumoto K., Tamura S. and Fujiki Y. (1998) Clofibrate-inducible, 28-kDa peroxisomal integral membrane protein is encoded by *PEX11*. FEBS Lett. **431:** 468–472