1. Introduction

In the early 1950s, peroxisomes were first identified as subcellular structures without any specific function. In 1966 De Duve and Baudhuin (Baudhuin, 1966) discovered, during their studies of the enzymes involved in the metabolism of glucose in liver cells by using differential and density-gradient centrifugation. They showed that the newly discovered organelles contain hydrogen peroxide-producing enzymes (glycolate oxidase, urate oxidase and D-amino acid oxidase) as well as H_2O_2-degrading enzyme (catalase). Due to the high content of hydrogen peroxide in the new organelles, these organelles were named –'peroxisomes'.

In the 1980s, the importance of peroxisomes in human metabolism was revealed by the correlation between impaired peroxisomes and the inborn disease - Zellweger syndrome (Lazarow et al., 1985; Santos et al., 1988a), which is connected to abnormalities in the fatty acid profile and plasmalogens.

1.1 Peroxisomes

Peroxisomes are single-membrane-bound vesicles, which can vary in size from 0.1-1 µm (Lazarow PB, 1985) and appear in different shapes such as spherical, elongated, tubular and reticular (Lazarow et al., 1985; Schrader et al., 1998). One of the most important peroxisomal biochemical processes is the β-oxidation of long and very long chain fatty acids. As mentioned above, peroxisomes also contain the enzyme catalase, which degrades hydrogen peroxide produced by β-oxidation and other peroxisomal pathways. In humans, peroxisomes are the site of synthesis of ether-linked phospholipids and sterols as well as the α-oxidation of long, β-methyl-branched chain fatty acids such as phytanate and the α-oxidation of other fatty acids and small molecules such as glycolate (Barth et al., 2001).

Peroxisomes are not only of different sizes and shapes but they are also unusually diverse organelles containing very different sets of enzymes, even in different cells of the same organism. They also can adapt remarkably to changing conditions. Yeast cells grown on sugar, for example, have small peroxisomes. But when some strains of yeast are grown on methanol, they develop large peroxisomes that oxidize methanol;

and when grown on fatty acids, they develop large peroxisomes that break down fatty acids to acetyl CoA by β-oxidation. This character of peroxisomes in yeast was successfully exploited as a general method to induce peroxisomes in appropriate studies by shifting yeast cells grown on glucose medium to certain mediums containing fatty acids, for example oleic acid, as exclusive carbon source (Erdmann et al., 1989; Veenhuis et al., 1987).

A major function of the oxidative reactions carried out in peroxisomes is the breakdown of fatty acid molecules. Acetyl CoA as a product of β-oxidation is exported from peroxisomes to the cytosol as energy carrer for reuse in biosynthetic reactions. β-oxidation in mammalian cells occurs both in mitochondria and peroxisomes; In yeast and plant cells, however, this essential biochemical reaction has been exclusively found in peroxisomes.

Catalase utilizes the H_2O_2 generated by other enzymes in the organelle to oxidize a variety of other substrates – including phenols, formic acid, formaldehyde, and alcohol – by the "peroxidative" reaction: $H_2O_2 + R'H_2 \rightarrow R' + 2H_2O$. This type of oxidative reaction is particularly important in liver and kidney cells, whose peroxisomes detoxify various toxic molecules that enter the bloodstream. About a quarter of the ethanol we drink is oxidized to acetaldehyde in this way. In addition, when excess H_2O_2 accumulates in the cell, catalase converts it to H_2O ($2\ H_2O_2 \rightarrow 2H_2O + O_2$)

1.2 Peroxisomal Disorders

Defects in either a single or more peroxisomal enzymes or in peroxisome biogenesis can cause peroxisomal disorders, which in turn often results in some human diseases. This was shown first time by the studies of correlation between the disease Zellweger Syndrom and peroxisomal disorders (Goldfischer et al., 1973). Peroxisomal disorders can be divided into three groups: Group I of these diseases covers all diseases characterized by the complete loss of general peroxisomal functions, including Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), and infantile Refsum disease (IRD) (Braverman et al., 1995; Lazarow and Moser, 1995; Moser et al., 1995). However ghost peroxisomes – peroxisome remnants – can be found in the

cell of these patient (Santos et al., 1988b); Group II consists of all diseases caused by the partial loss of peroxisomal functions, for example partial defects of import of peroxisomal matrix proteins. The rhizomelic chondrodysplasia punctata (RCDP) disease clinically characterized by rhizomelia, dysmorphic facial feature and abnormal psychomotor development (Geisbrecht and Gould, 1999; Lazarow, 1995) belongs to this group. RCDP is caused by a defect in branched chain fatty acid metabolism and by a defect in plasmalogen synthesi.; Group III of peroxisomal disorders is caused by a defect of a single peroxisomal enzyme, for example X-linked adrenoleukodystrophy (X-ALD) and Refsum's disease. X-ALD is caused by a defect in a peroxisome membrane protein (Mosser et al., 1994), which is an ATP dependent molecular transporter. X-ALD is reflected by the accumulation of very long chain fatty acids in plasma and tissue and is clinically registered by progressive demyelination of the central nervous system. Refsum's disease is caused by defects in a protein required for metabolism of phytanic acid and phytanoyl-CoA hydroxylase resulting in elevated levels of phytanic acid in plasma and tissue, retinitis pigmentosa, peripheral neuropathy and cerebellar ataxia (Sacksteder and Gould, 2000).

1.3 PEX-Genes, Peroxins and Mutants with Defects in Peroxisomal Biogenesis

PEX-genes encode such proteins – peroxins, which are essential for the peroxisomal biogenesis; up to date there are 25 peroxins identified in different organisms (see table 1). If one or more than one of these PEX-genes are mutated or knocked out, peroxisomes are defect in their proliferation or biogenesis. Characteristics of these mutants help us also understand peroxisomal diseases.

It was shown (Veenhuis et al., 1987) that β-oxidation of lipids in yeast *Saccharomyces cerevisiae* takes place exclusively in peroxisome, this made it possible to isolate peroxisomal mutants of yeast *Saccharomyces cerevisiae* by growing *S.c.* yeast in oleate medium. These peroxisomal mutants, named as onu-mutants(**o**leate-**n**on-**u**tilizer), which have a complete defect in β-oxidation of fatty acids, can be divided into two groups: fox-mutants(**f**atty acid **ox**idation), which have a defect in one of the β-oxidation enzymes; pex-mutants(**pe**ro**x**isome assembly), which have no peroxisomes or defect in peroxisomal protein import.

Table 1: PEX-genes

Gen	Peroxin-characteristics	Org.	References
PEX1	AAA-family ATPase	*Sc* *Pp* *Hs*	*(Erdmann et al., 1991)* *(Heyman et al., 1994)* *(Reuber et al., 1997);* *(Portsteffen et al., 1997)*
PEX2	C3HC4 zinc-binding integral peroxisomal membrane protein; 35-52 kDa; mutations responsible for complementation group10 of the PBD.	*Rn* *Hs* *Pp* *Sc*	*(Tsukamoto et al., 1991)* *(Shimozawa et al., 1992)* *(Waterham et al., 1996)* *(Erdmann and Kunau, 1992)*
PEX3	51-52 kDa integral peroxisomal membrane protein lacking similarity to other proteins	*Sc* *Hp* *Pp* *Hs*	*(Hohfeld et al., 1991)* *(Baerends et al., 1996)* *(Wiemer et al., 1996)* *(Kammerer et al., 1998)*
PEX4	ubiquitin-conjugating enzyme	*Sc* *Pp* *Hp*	*(Wiebel and Kunau, 1992)* *(Crane et al., 1994)* *(van der Klei et al., 1998)*
PEX5	PTS1 receptor; 64-69 kDa protein containing 8-9 tetratricopeptide repeats; localized to the cytoplasm and peroxisome; mutations responsible for complementation group 2 of the PBD	*Pp* *Sc* *Hs* *Hp* *Yl*	*(McCollum et al., 1993)* *(van der Leij et al., 1993)* *(Dodt et al., 1995; Fransen et al., 1998; Wiemer et al., 1995)* *(Nuttley et al., 1995; van der Klei et al., 1995)* *(Szilard et al., 1995)*
PEX6	Belongs to the AAA family of ATPases; 112-127 kDa; localized to cytoplasm and peroxisome; mutations responsible for complementation group 4 of the PBD.	*Pp* Sc *Yl* *Rn* *Hs*	*(Spong and Subramani, 1993)* *(Voorn-Brouwer et al., 1993)* *(Nuttley et al., 1994)* *(Tsukamoto et al., 1995)* *(Yahraus et al., 1996)*
PEX7	PTS2 receptor; 42 kDa protein containing six WD40 repeats; localized to the cytosol and peroxisome; mutations responsible for complementation group 11 of the PBD	*Sc* *Hs* *Pp*	*(Marzioch et al., 1994)* *(Braverman et al., 1997; Motley et al., 1997; Purdue et al., 1997)* *(Elgersma et al., 1998)*
PEX8	71-81 kDa peroxisome-associated protein containing a PTS1 signal.	*Hp* *Pp* *Yl* *Sc*	*(Waterham et al., 1994)* *(Liu et al., 1995)* *(Liu et al., 1995)* *(Erdmann and Kunau, 1992)*
PEX9	42 kDa integral peroxisomal membrane protein lacking similarity to other proteins.	*Yl*	*(Eitzen et al., 1995)*
PEX10	C3HC4zinc-binding integral peroxisomal membrane protein; 34-48 kDa.	*Hp* *Pp* *Hs* *Sc*	*(Tan et al., 1995)* *(Kalish et al., 1996)* *(Warren et al., 1998)* *(Erdmann and Kunau, 1992)*
PEX11	27-32 kDa peroxisome-associated	*Sc*	*(Erdmann and Blobel, 1995)*

	protein involved in peroxisome proliferation	*Hs*	*(Abe et al., 1998)*
PEX12	48 kDa C3HC4 zinc-binding integral peroxisomal membrane protein	*Pp* *Hs* *Sc*	*(Kalish et al., 1996)* *(Chang et al., 1997)* *(Erdmann and Kunau, 1992)*
PEX13	SH3-containing, 40-43 kDa integral peroxisomal membrane protein; binds the PTS1 receptor and PEX14p	*Sc* *Pp* *Hs*	*(Elgersma et al., 1996);* *(Erdmann and Blobel, 1996)* *(Gould et al., 1996)* *(Gould et al., 1996)*
PEX14	38 kDa peroxisome associated protein, binds both PTS1 and PTS2 receptor and Pex13p-SH3	*Hp* *Sc* *Hs*	*(Komori et al., 1997)* *(Brocard et al., 1997);* *(Albertini et al., 1997)* *(Fransen et al., 1998)*
PEX15	44 kDa phosphorylated integral peroxisomal membrane protein, no homolog in other organism	*Sc*	*(Elgersma et al., 1997)*
PEX16	44 kDa peripheral protein located at the matrix face of the peroxisomal membrane, no homolog in other organism	*Yl* *Hs*	*(Eitzen et al., 1997)* *(South and Gould, 1999)*
PEX17	23 kDa peroxisome associated protein, binds Pex14p	*Sc*	*(Huhse et al., 1998)*
PEX18	66 kDa protein containing a PTS2, disruption results in abbarent peroxisome morphology	*Sc*	*(Purdue et al., 1998)*
PEX19	40 kDa farnesylated protein associated with peroxisomes	*Hs* *Sc*	*(Braun et al., 1994)* *(Gotte et al., 1998)*
PEX20	cytosolic, interact with Pex8p, involved in import of thiolase	*Yl*	*(Titorenko et al., 1998)* *(Einwachter et al., 2001b)* *(Smith and Rachubinski, 2001)*
PEX21	Bindes to Pex7p	*Sc*	*(Purdue et al., 1998)*
PEX22	The fisrt 20 residues contains mPTS, interact with Pex4p	*Pp*	*(Koller et al., 1999)*
PEX23	related to import of peroxisomal matrix proteins	Yl	*(Brown et al., 2000)*
PEX24	integral membrane protein,		*(Tam and Rachubinski, 2002)*
PEX25	required for the regulation of peroxisome size and maintenance		*(Smith et al., 2002)*

1.4 Peroxisomal Biogenesis

Early theories about peroxisome biogenesis supported the model in which peroxisomes are formed by fusion of vesicles associated with ER. This model has also been proposed in experiments with *Yarrowia lipolytica* (Titorenko et al., 1997; Titorenko and Rachubinski, 1998a) showing that cells defective in protein secretion are impaired in peroxisomal biogenesis. In addition, studies in *Saccharomyces*

cerevisiae demonstrated that some truncated peroxisomal membrane proteins (PMP) are localized in the ER suggesting that these PMPs pass through the ER before being transported to peroxisomes (Blobel and Erdmann, 1996; Elgersma et al., 1998; Gotte et al., 1998). Furthermore, experiments with *Hansenula polymorpha* showed that inhibition of COPI vesicles results in accumulation of peroxisomal proteins in the ER (South et al., 2000). The second pathway of peroxisome biogenesis has been proposed by Lazarow and Fujiki (Lazarow et al., 1985), according to which peroxisomes are formed by growth and division. This model suggests that peroxisomes are formed by fission of pre-peroxisomes after the import of newly synthesized proteins from the cytosol. However, formation of peroxisomes in cells lacking detectable peroxisomes cannot be explained by this model opening the possibility that peroxisomes can arise '*de novo*' (Erdmann et al., 1997). Formation of peroxisomes '*de novo*' includes assembly of the import machinery for peroxisomal membrane proteins (PMP) and matrix proteins followed by formation of pre-peroxisomes. After the import of peroxisomal proteins from cytosol, peroxisomes presumably mature into large, functional organelles.

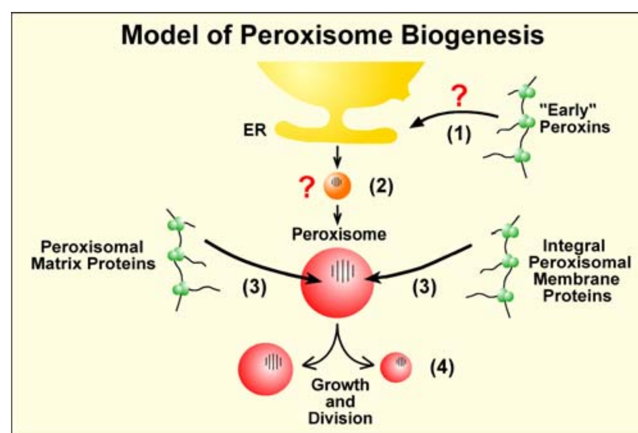

Fig. 1 Peroxisome biogenesis models (Holroyd and Erdmann, 2001)

1.5 Import and Synthesis of Peroxisome Membrane Proteins

Peroxisome membrane proteins (PMP's) are synthesized on free polyribosomes and their targeting to the peroxisomal membrane occurs independently of the import mechanism of matrix proteins (Baerends et al., 2000; Erdmann and Blobel, 1996; Gould et al., 1996). In fact, PMP's are sorted to the peroxisomal membrane by targeting signals (mPTS), which are distinct from the PTS1 and PTS2 targeting

signals of matrix proteins. mPTSs are found in close proximity to hydrophobic regions of different protein domains such as C-terminal, N-terminal and internal domain (Snyder et al., 2000; Subramani et al., 2000) (refer to the fig. 2).

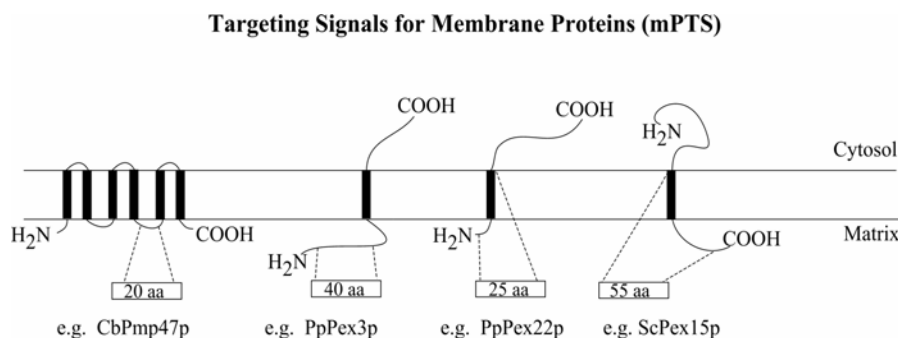

Fig. 2 Targeting signal for membrane proteins (Subramani et al., 2000)

Based on the function of their target proteins, mPTSs could be divided in two different classes:

(1) mPTSs which deliver proteins from the cytosol directly to the peroxisomal membrane,

(2) mPTSs which direct proteins involved in peroxisome biosynthesis to peroxisomes via the ER.

Cells lacking genes responsible for production of PMP's are characterized by absence of visible peroxisomal structures. Cellular studies in *S. cerevisiae* have shown that mutations in the PEX3 and PEX19 genes affect drastically PMP import and some other pathways of peroxisomal membrane biogenesis (Hettema et al., 2000).

1.6 Import matrix proteins into peroxisomes

Most peroxisomal proteins are synthesized on free polyribosomes in the cytosol and transported post-translationally into the peroxisomes. Subramani and coworkers showed that protein transport into peroxisomes is mediated by two types of signals, PTS1 and PTS2 (Fig.3) (Brocard et al., 1994; Dodt et al., 1995; Fransen et al., 1995; McCollum et al., 1993; Osumi et al., 1991; Subramani et al., 2000; Swinkels et al., 1991; Terlecky et al., 1995). The targeting signals are recognized by their appropriate receptors in the cytoplasm followed by docking of the receptor and cargo at the peroxisomal membrane, translocation of cargo into peroxisomal membrane and recycling of the receptor to the cytoplasm.

1.6.1 Peroxisomal targeting signals

One of the most common peroxisomal targeting signals is PTS1, which consists of the C-terminal tripeptide (Ser-Lys-Leu) sequence. The PTS1 sequence varies in different species and it influences the protein conformation (Erdmann et al., 1997). The second targeting signal, PTS2, consists of an internal or N-terminal nonapeptide with the consensus sequence of Arg/Lys-Leu/Ile-5x-His/Gln-Leu (Swinkels et al., 1991).

Targeting Signals for Matrix Proteins

-PTS1 - a C-terminal tripeptide (e.g. luciferase)

-PTS2 - a N-terminal nonapeptide (e.g. thiolase)

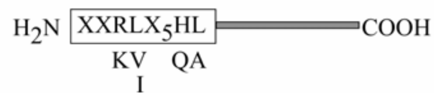

Fig. 3 Targeting signals used by peroxisomal proteins. The PTS sequences are marked in the boxes and their variants are shown below these sequences (Subramani et al., 2000).

1.6.2 Recognition Receptors of Peroxisomal Matrix Proteins

The peroxisomal targeting signals, PTS1 and PTS2, are recognized by cytosolic signal-recognition factors, Pex5p and Pex7p respectively. Pex5p is a receptor for PTS1 and Pex7p is a recognition receptor for PTS2. The Pex5p receptor contains 6 or 7 tetratricopeptide repeats (TPRs) in its C-terminal domain (Parsons et al., 2001). Mutation analysis of Pex5p has revealed that TPR domains form two clusters of three TPR's motifs connected with a single TPR sequence. In fact, these two clusters are placed close to each other forming a single binding site for PTS1 (Das et al., 1998). In mammals, two isoforms of the peroxisome targeting signal (PTS) type 1 receptor Pex5p, i.e. Pex5pS and Pex5pL with an internal 37-amino acid insertion, have previously been identified, of which the shorter form, Pex5pS, interacts with PTS1 and the longer form, Pex5pL, interacts with PTS1 and PTS2 via Pex7p . In addition, it was found that Pex5L binds directly to the Pex7p-PTS2 complex carrying its cargo PTS2 protein in the cytosol (Otera et al., 2000) and disruption of the Pex5pL-Pex7p interaction prevents PTS2 protein import in mammals (Matsumura et al., 2000). As mentioned above, targeting of PTS2 proteins is mediated by the Pex7p receptor. All Pex7p proteins are characterized by WD motifs which consist of 44-60 aa sequence and a conserved Trp-Asp dipeptide (Rehling et al., 1996)

Additional factors required for import of PTS2 proteins are two structurally related proteins, Pex18p and Pex21p in *S. cerevisiae* (Einwachter et al., 2001a) and Pex20p in *Y. lipolytica (Titorenko et al., 2000)*. The Pex18p and Pex21p show specific homology to each other and partial functional redundancy, and both interact with Pex7p (Purdue et al., 1998). Absence of either of the proteins has only a modest defect on PTS2 import, whereas lack of both proteins abolishes the PTS2 pathway. Interestingly Pex20p is the only necessary but also sufficient receptor protein required for import of thiolase, a PTS2 targeting protein, in *Y. lipolytica* (Titorenko et al., 1998).

1.6.3 Role of molecular chaperons in protein import

Molecular chaperones are involved in different processes such as protein folding, disassembly of oligomeric protein complexes, delivery of proteins to proteases and translocation of polypeptides across intracellular membranes. Studies in *S. cerevisiae* showed that in the absence of cytosolic J-domain-containing protein – Djp1p, peroxisomal matrix proteins mislocalize partially to the cytosol (Hettema et al., 1998). The role of Djp1p in peroxisomal matrix protein import has not been confirmed yet, but it is suspected that Djp1p keeps newly synthesized proteins in the conformation optimal for translocation. In addition, it has been suggested that Djp1p stimulates recognition of peroxisomal proteins by their appropriate receptors (Hettema et al., 1999).

1.6.4 Membrane Docking Machinery

The receptors for peroxisomal matrix proteins, after loaded with their cargo protein – the peroxisomal matrix protein to be translocated, land on the docking machinery embedded in the peroxisomal membrane, where the PTS-containing protein is transferred into the next step of import to the peroxisomal matrix.

Membrane docking complex consists of at least one integral protein, Pex13p, and two membrane-associated proteins, Pex14p and Pex17p. In addition to the membrane-docking complex, three other peroxins take part in PTS import pathway: Pex10p, Pex12p and Pex4p.

Pex13p, with C- and N- termini extending into the cytosol, is the key component protein in the docking machinery (Girzalsky et al., 1999) and shows interaction with another component, Pex14p, of the docking machinery and with the two import receptors, Pex5p and Pex7p. At the C-terminal of Pex13p is a Src homology 3 (SH3) domain (so called because it was first found in the Src protein), which directly interacts with the well characterized proline rich motif PXXP in Pex14p, however the structure details of this particular SH3-PXXP interaction were first studied in this work; Moreover, the SH3 domain of Pex13p is necessary for its binding to one of the peroxisomal receptors Pex5p (Erdmann and Blobel, 1996), even though it lacks a typical binding motif PXXP for SH3. Furthermore, Pex13p binding regions of Pex5p and Pex14p do not overlap (Girzalsky et al., 1999). Studies of the Pex7p and Pex13p interaction (Girzalsky et al., 1999) confirmed whether this interaction is direct (Stein et al., 2002). Yeast two-hybrid and co-immunoprecipitation experiments suggest that the N-terminal domain of Pex13p (not the C-terminal SH3 domain) binds to Pex7p.

Pex14p is peripherally associated with the peroxisomal membrane in yeast, while it appears as an integral protein in mammals (Shimizu et al., 1999). One of the binding sites of Pex14p is the PXXP motif, which binds to the C-terminal SH3 domain of Pex13p (Girzalsky et al., 1999). Furthermore, it has been shown that Pex14p interacts not only with Pex13p but also with Pex5p and Pex17p. The interaction of Pex14p and Pex5p is involved in the peroxisomal matrix protein import, while the interaction between Pex14p and Pex3p or Pex17p is important for association of Pex14p to the peroxisomal membrane (Subramani et al., 2000).

Pex17p is a protein with a single membrane domain. In yeast it's a peripheral membrane protein. C-terminus of Pex17p is localized in the cytosol and the N-terminus is facing peroxisomal matrix (Snyder et al., 1999b). Pex17p is important not only for the import of peroxisomal proteins but also for the import of peroxisomal membrane proteins (PMP) such as Pex3p and Pex22p (Snyder et al., 1999a). The importance of Pex17p for the import of some PMP's was shown in the tests of *pex17*Δ mutants, which were characterized for impaired transport of matrix proteins (Snyder et al., 1999a). In addition to the proteins of membrane docking complex described above, following proteins take part in the import of matrix proteins: Pex2p, Pex10p,

Pex12p (Subramani, 1998), Pex15p (Elgersma et al., 1997), Pex22p (Koller et al., 1999) and Pex23p (Brown et al., 2000).

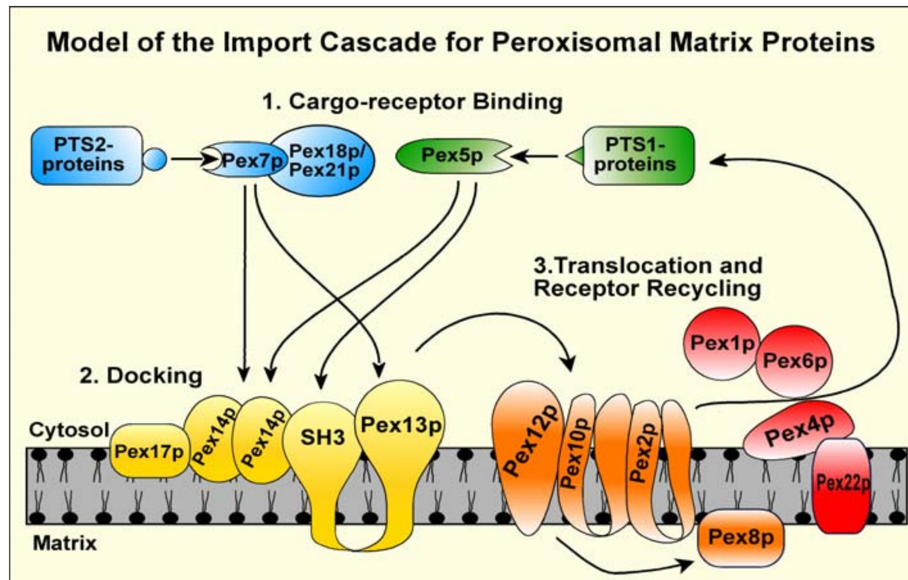

Fig. 4 Model for the import cascade of peroxisomal matrix proteins (Holroyd and Erdmann, 2001)

1.6.5 Translocation and receptor recycling

The mechanism of translocation of peroxisomal proteins and subsequent recycling of their import receptors has not been experimentally confirmed. The three translocation models are suggested up to now include translocation through: a pore, a channel and pinching off peroxisomal membrane vesicles (Dammai and Subramani, 2001; Purdue and Lazarow, 2001). Electron microscopic experiments showed no sign of a pore of a similar size as nuclear pore excluding the option of protein translocation through a pore. The existence of peroxisome ghosts with multiple membrane layers and peroxisomes with internal membrane structures (McNew and Goodman, 1994) support the idea of peroxisomal protein import via endocytotic-like intermediates. Translocation through a channel would require large channels, which could transport oligomers and PTS- bearing –9nm-gold particles. In addition, channels would need to be gated because the peroxisomal membrane is impermeable to many metabolites (van Roermund et al., 1995) and because high pH gradient between cytosol and peroxisomal matrix has to be retained (Nicolay et al., 1987).

The peroxisomal translocation system can transport proteins in different forms: folded proteins, proteins cross-linked to a PTS protein (Walton et al., 1995) and oligomers, which are properly folded in the cytosol before they are transported across the membrane. It is not known whether the PTS receptor dissociates prior or after peroxisomal proteins are translocated into the peroxisomes. Therefore two translocation models have been proposed:
(1) translocation of protein-receptor complex is followed by dissociation of the receptor, which shuttles back to the cytoplasm.
(2) docking of protein receptor-complex is accompanied by dissociation of the receptor and by translocation of the PTS containing protein alone across the peroxisomal membrane.

Import of peroxisomal matrix proteins is supposed to be mediated by the following RING finger peroxins: Pex2p, Pex10p and Pex12p. All three peroxins are integral membrane proteins which function downstream of the docking complex (Chang et al., 1999). In addition, Pex4p and Pex22p are required for import of PTS1 and PTS2 proteins except for PTS2 proteins in *H. polymorpha* (van der Klei et al., 1998). Pex4p is a peroxisomal ubiquitin-conjugating enzyme (Wiebel and Kunau, 1992), which is anchored to the cytosolic site of the peroxisomal membrane by interaction with the integral membrane protein Pex22p. Although the role of Pex4p and Pex22p has not been confirmed yet, it is suggested that Pex4p/Pex22p complex and the RING proteins, Pex12p/Pex10p/Pex2p, take part in modification of the import receptors of matrix proteins before their translocation or receptor recycling (Holroyd and Erdmann, 2001).

PEX1 and PEX6 encode proteins which belong to the AAA ATPases group (Faber et al., 1998). Some studies have indicated that Pex1p and Pex6p are involved in the fusion of vesicles derived from endoplasmatic reticulum (ER) (Titorenko and Rachubinski, 1998b). Other studies have proposed that Pex1p and Pex6p play a role in matrix protein import (Dodt and Gould, 1996). Evidence of AAA ATPase role in assembly, organization and disassembly of protein complexes (Neuwald et al., 1999) supports the idea that Pex1p and Pex6p participate in matrix protein import rather than in peroxisome membrane biogenesis.

1.7 Yeast as a Model System for Studying Peroxisomes

Taking into consideration that basic principles of peroxisome biogenesis are conserved between yeast and higher eukaryotes, yeast was presented as a suitable model organism for studies of this process (Erdmann et al., 1997). Yeast peroxisomes are the only site of fatty acid β-oxidation and expression of β-oxidation enzymes could be regulated by exposure of cells to various carbon sources. Therefore, most genes encoding peroxisomal proteins are induced in the presence of fatty acids such as oleate. In addition, some yeast strains can use methanol in their peroxisomal pathways enabling growth tests of yeast peroxisome mutants on methanol (Trotter, 2001). Mutations in peroxisome biogenesis are studied using different yeast strains, such as *Saccharomyces cerevisiae*, *Pichia Pastoris*, *Hansenula polymorpha* and *Yarrowia lipolytica* (Distel et al., 1996a; Distel et al., 1996b). Beside the studies of peroxisome biogenesis, localization of peroxisomal matrix enzymes and presence or absence of organelles in peroxisome biogenesis mutants can be identified by fluorescent and electron microscopy (Erdmann et al., 1989).

1.8 Setting of Tasks in this Work

This work focused on the import of the peroxisomal matrix proteins with PTS1 targeting signal:

- Localization of the binding site of ScPex5p in ScPex13p
- Localization of the binding site of ScPex13p in ScPex5p
- Localization of the binding site of ScPex14p in ScPex13p
- Localization of the binding site of ScPex13p in ScPex14p
- Characterization of the interactions among Pex5p, Pex13p (SH3 domain) and Pex14p.
- Characterization of the ScPex13p SH3 domain and illustration of its structure by NMR spectroscopy.
- Illustrustion of the docking process of the cargo complex consisting of the PTS1 proteins and their receptor Pex5p on the peroxisomal membrane.