Peroxisome Biogenesis and Positioning.

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Abstract
Plant peroxisomes are extremely dynamic, moving and undergoing changes of shape in response to metabolic and environmental signals. Matrix proteins are imported via one of two import pathways depending upon the targeting signal within the protein. Each pathway has a receptor but utilize common membrane bound translocation machinery. Current models invoke receptor recycling, which may involve cycles of ubiquitination. Some components of the import machinery may also play a role in proteolytic turnover of matrix proteins, prompting parallels with the ERAD pathway for degradation of ER proteins. Peroxisome membrane proteins, some of which are imported post translationally, others of which may traffic to peroxisomes via the endoplasmic reticulum, use distinct proteinaceous machinery. Isolation of mutants defective in peroxisome biogenesis has served to emphasize the important role of peroxisomes at all stages of the plant lifecycle.

Introduction

The development of *in vivo* imaging techniques has led to a growing appreciation that organelles are extremely dynamic, often pleomorphic, and motile structures (Movie 1). Peroxisomes are fascinating organelles that are major players in pathways of carbon metabolism in plants including mobilization of seed storage oils, turnover of membrane lipids and photorespiration, as well as being an important source of signaling molecules. While peroxisomes (visualized by the targeting of a fluorescent protein reporter) are generally visible as punctuate bodies of 1-2 μm in diameter in all cell types, size and appearance can vary. For example large peroxisomes are present in clusters at the onset of seed germination and sometimes buds, tails or loops are seen (Figure 1).

Peroxisome positioning

Peroxisome morphology and position are most likely influenced by metabolic signals. In onion cells peroxisomes alter their location during the cell cycle. In interphase peroxisomes are randomly distributed within the cell, but during late anaphase they align along the division plane and by early telophase have divided into two lines either side of where the new cell plate will form. By late telophase the peroxisomes segregate into two groups at the inner edge of the phragmoplast and then begin to distribute between the daughter cells [1]. Similar observations were made in other monocots but no evidence for alignment of peroxisomes during cell division in Arabidopsis was seen [1]. The purpose of this behavior is not known but it was speculated that some metabolic function of peroxisomes e.g. β-oxidation or hydrogen peroxide production could be important in the formation of the new cell plate.

A second example of peroxisome movement occurs in response to powdery mildew fungal infection. Peroxisomes and other organelles accumulate at the site of penetration of the fungal haustoria [2]. Mechanical stimulus alone seems to be sufficient for the organelle migration to the point of attempted fungal penetration and occurs within three to five minutes of touching the plant surface with a needle [3]. The *pen2* mutant was isolated in a screen for Arabidopsis mutants that allowed efficient entry of the grass powdery mildew fungus *Blumeria graminis f sp. hordei* which is not normally able to colonize Arabidopsis. The *PEN2* gene encodes a peroxisomal localized glycosyl hydrolase and a mutation predicted to confer loss of catalytic activity resulted in a *pen2* phenotype. It was hypothesized that the accumulation of PEN2-containing peroxisomes at the invasion site could result in PEN2 either directly or indirectly producing a small molecule that has broad spectrum activity against fungal pathogens [4].

Peroxisome movement, like that of other plant organelles is driven by the actinomyosin system which is discussed in more detail in the accompanying article by Sparkes.

Variation in peroxisome morphology

Two recent papers provide evidence that peroxisomes alter their morphology and movement in response to reactive oxygen species. Rodríguez-Serrano et al., [5] showed that Arabidopsis plants grown in the presence of 100 μM cadmium had lower levels of Cu-Zn superoxide dismutase and higher levels of superoxide radical . In these plants one to two days post-treatment, peroxisomes moved more rapidly and this effect was largely prevented by the inclusion of the scavenger ascorbate. Infiltration of leaves with xanthine (which produces intraperoxisomal reactive oxygen

species from xanthine oxidase) also causes more rapid movement of peroxisomes, whilst induction of hydrogen peroxide outside of peroxisomes by glucose/glucose oxidase or direct addition of H_2O_2 had no effect. Peroxisomes often extend long tubular processes termed 'peroxules' [6] (Figure 1, Movie 1). A recent study [7] showed that peroxules were rapidly (within seconds) produced in response to hydrogen peroxide or UV light treatments but not by singlet oxygen. Peroxule extension did not appear to involve the cytoskeleton, but peroxules appeared to co-align with ER tubules. The mechanisms and reasons for these observations remain unknown, although roles in metabolite or protein transfer have been proposed [6, 7].

A number of genetic mutants show alteration of peroxisome morphology. Mutants affecting peroxisome division and segregation will not be discussed here but the reader is referred to the accompanying review by Hu.

Several mutants in peroxisomal β-oxidation show enlarged peroxisomes. This effect has been reported in the *kat2/ped1* mutant defective in the major seedling 3-ketoacyl thiolase and the *mpf2* mutant defective in the multifunctional protein. Since mutants in the fatty acid transporter CTS and the peroxisomal acyl CoA synthetases LACS6 and LACS7 do not have enlarged peroxisomes, it has been proposed that intraperoxisomal accumulation of acyl CoAs may be the signal for peroxisome enlargement, although direct proof is lacking [8].

Identification of peroxisome protein import mutants in plants.
Two successful screens for peroxisome deficient mutants have been developed which rely on peroxisomal β-oxidation to convert the pro-auxins indole butyric acid (IBA) and 2,4-dichloro-phenoxy-butyric acid (2,4-DB) to active auxins (IAA and 2,4-D respectively) [9, 10]. Plants sensitive to these compounds have short roots, whereas resistant mutants have long roots when grown on the pro-auxin but retain sensitivity to IAA or 2,4-D. Resistance occurs due to failure to convert the pro-auxin to the active form; this can be due to defects in the enzymes required for metabolism or in their correct localization to peroxisomes. An alternative strategy has taken advantage of the availability of the genome sequence and collections of insertion mutants of Arabidopsis to search for homologues of genes implicated in peroxisome biogenesis in mammals and yeast [11]. Table 1 presents a compilation of mutants identified by these and other approaches.

Matrix protein import.
Two soluble protein receptors recognize cytosolically synthesized peroxisomal matrix proteins. PEX5 binds to a carboxy terminal tripeptide called PTS1. This tripeptide generally conforms to the pattern: ′small side chain′-′basic side chain′-′hydrophobic side chain′-COOH, where COOH is the C-terminal carboxylate group. The X-ray structure of the PTS1 binding domain of human PEX5 bound to a model peptide (YQSKL) shows that PEX5 provides a binding pocket lined with appropriate hydrogen bond donors and acceptors to recognize the PTS1 peptide [12].

Bioinformatic and proteomic approaches have produced a catalogue of likely peroxisomal proteins [13] which have been tested in some cases by fusion to reporter proteins such as GFP [14]. These studies show that plant PTS1s largely conform to the expected consensus, with SKL being the most frequently occurring PTS1 in the Arabidopsis genome, followed by SRL and SRI [15].

PEX7 is the second soluble receptor which binds to the PTS2 sequence [16]; a nonapeptide found towards the N terminus of a smaller number of peroxisomal proteins including the 3-ketoacyl thiolases, peroxisomal malate dehydrogenase and citrate sythnase. The most frequent PTS2 sequences in Arabidopsis are RLX_5HL and RIX_5HL where X is any amino acid. Interestingly the thiolases all have a variant RQX_5HL [15]. There is currently no structural information on the interaction between PTS2 and PEX7.

Arabidopsis mutants deficient in PEX7, as expected, show defects in PTS2 matrix protein import (Table 1), [17, 18]. Down regulation of PEX5 by RNAi resulted in a defect in the import of both PTS1 and PTS2 proteins [17] and the interaction of Arabidopsis PEX5 and PEX7 in the yeast two hybrid system [16] suggests that PEX5 is required as a co-receptor for PEX7 in plants as it is in mammals [19]. Interestingly the mutation in the *pex5-1* allele (Table 1) results in loss of PTS2 import only and is in the same conserved serine residue as a mutant in PEX5 from Chinese hamster ovary (CHO) cells which are also deficient in PTS2 import only [19]. The *pex5-1* and *pex7* mutants show strong seedling phenotypes (Table 1). They are sucrose dependent for post-germinative growth and resistant to IBA and 2,4-DB, indicative of defective β-oxidation. However adult plants are much less severely affected than many other *pex* mutants. This is consistent with the relatively high proportion of β-oxidation enzymes targeted by the PTS2 import route (Figure 2).

AtPEX14 is an integral peroxisomal membrane protein [20]. It interacts with AtPEX5 in the yeast two hybrid system [16], and antibodies raised against human PEX14 immunoprecipitate Arabidopsis PEX14 and block the binding of PTS1 and PTS2 proteins to isolated peroxisomes [21]. These observations are consistent with the role of PEX14 as a docking protein that receives cargo-loaded PEX5 at the peroxisome. A mutant *ped2* defective in AtPEX14 (Table 1) was isolated using the 2,4-DB resistance screen [10]. The *ped2* mutant shows a partial defect in both PTS1 and PTS2-mediated protein import and the peroxisomes are small and irregular shaped, consistent with reduced matrix content [20]. The *ped2* mutant is pale and dwarfish when grown in a normal atmosphere and this phenotype is recovered upon growth in elevated CO_2, indicative of a photorespiratory defect.

PEX13 is also a member of the docking complex in yeast and mammals and the *pex13* mutant *apm2* was isolated from a screen for mutants that mislocalize a GFP-PTS1 reporter to peroxisomes in Arabidopsis [22].The mutation introduces a premature stop codon, thereby deleting the last 40 amino acids of the protein. Mutants still show some GFP fluorescence in peroxisomes and display relatively mild growth defects; the mutant can establish without sucrose and has only slight 2,4-DB resistance (Table 1). PTS2 import is also compromised, but not completely abolished [22]. The N terminal portion of AtPEX13 interacts with AtPEX7 in the yeast two hybrid assay [22] thus the truncated protein, if stably integrated in the peroxisome membrane, may have partial activity, explaining the relatively mild phenotype. More recently a new allele of *pex13* was isolated as a mutant in which pollen tube perception by the female gametophyte is impaired [23]. This mutant was named *abstinence by mutual consent* (*amc*) as failure of fertilization only occurred when both male and female gametes carry the *amc* mutant allele. The mutation is caused by a T-DNA insertion within the PEX13 gene, therefore it is likely to be a null mutant. Finally the *amc* mutant was demonstrated to disrupt PTS1 protein import into

pollen peroxisomes [23]. These results show that successful pollen tube perception and fertilization requires functional peroxisomes in both gametes. The reason for this is unclear although the authors speculate that a peroxisome derived signaling molecule may play an important role in signaling between the gametes.

Once PEX5 and PEX7 receptors dock at the peroxisome membrane, the cargo is translocated to the lumen and unloaded (Figure 3A). The mechanism by which this occurs is unclear, reflecting the lack of information about the temporal sequence of events during protein import. Models based on data from mammalian and yeast systems suggest that the receptors insert deeply into the membrane or perhaps even escort their cargo across before cargo unloading and receptor recycling occur [19]. It is unclear whether PEX5 itself or possibly as a complex with PEX14 (and maybe other peroxins) forms the actual translocation channel.

PEX2, PEX10 and PEX12 constitute the RING finger peroxins. The RING domain is a cross braced structure in which two Zn^{2+} ions are coordinated by the side chains of cysteine and/or histidine. RING domains are frequently found in E3 ubiquitin ligases where they serve to bring together the substrate and the E2 enzyme which attaches ubiquitin (a 76 amino acid protein) to target proteins to modulate their activity or stability.

Null mutants of all three RING peroxins are embryo lethal in Arabidopsis (Table 1) [24-27]. *pex10* and *pex12* mutant embryos were able to develop to the heart stage but then aborted [24, 25, 27], which is a different phenotype from that seen in the *amc* allele of *pex13* where fertilization did not occur [23]. Sub-lethal mutants have been isolated by EMS mutagenesis (*apm4/pex12*, [22]) and by RNAi (*pex2, pex10 and pex12*, [28], Brown, unpublished data). In all cases the mutants showed protein import defects. The *apm4* mutant showed a severe defect in import of the PTS1 and PTS2 targeted GFP reporters, and a correspondingly severe growth defect. [22]. *pex12i* plants also displayed defects in peroxisome protein import [25, 28]. *pex10i* mutants had severe developmental defects, strong impairment in both PTS1 and PTS2 import and failed to produce viable seed [28]. The first *pex2* mutant allele, *ted3,* was isolated as a suppressor of the *det1-1* mutant [26]. *DET1* is a regulator of photomorphogenesis and encodes a gene regulatory protein. Dark grown *det1-1* mutants look more like light grown plants with short hypocotyls and green expanded cotyledons. The *ted3* mutant suppresses these developmental defects and is a missense mutation (valine to methionine) in PEX2 just before the RING domain. Antisense *TED3* [26] and *PEX2* [28] produced small pale plants with reduced fertility [26] that were sucrose dependent, 2,4-DB resistant and had a protein import defect [28]. Interestingly the *det1-1* mutant also shows some features of peroxisome deficiency such as small peroxisomes, reduced levels of some peroxisomal proteins, sucrose dependency and slight resistance to 2,4-DB which was suppressed by the *ted3* mutation [26].

Arabidopsis *pex4-1* was isolated as a reduced IBA response mutant [29] (Table 1). It has reduced number of lateral roots, a long root on IBA and is sucrose dependent for hypocotyl growth in the dark, indicating reduced β-oxidation. Import of a GFP-PTS1 reporter appeared normal but there was reduced processing of thiolase, a PTS2 protein, suggesting that its import may be compromised. The encoded protein is 35% identical to *S. cerevisiae* Pex4p an E2 ubiquitin conjugating enzyme that has recently been shown to ubiquitinate Pex5p in yeast (Figure 3A) [30]. In yeast Pex4p is anchored to the peroxisome membrane by a membrane protein, Pex22p.

Using AtPEX4 as bait in a yeast two hybrid screen an interacting protein was isolated that had similar size and predicted topology to ScPex22p, and both AtPEX4 and AtPEX22 could complement the corresponding yeast mutants. The double *pex4-1/pex22-1* mutant gave a more severe phenotype than either alone supporting the notion that both are partial loss-of-function mutants that operate in the same pathway [29].

In yeast ubiquitinated Pex5p is extracted from the peroxisome membrane by the AAA proteins Pex1p and Pex6p (Figure 3A) [31]. Two types of ubiqutination on Pex5p occur; monoubiquitination on a conserved cysteine and mono- or polyubiquitination on two adjacent lysines (K18 and K24). After removal from the membrane by the AAA complex, monoubiquitinated pex5p is recycled and polyubiquitinated Pex5p is degraded by the proteasome. This is viewed as a mechanism for removing defective Pex5p from the membrane, where it might otherwise accumulate and block further rounds of import (Figure 3A). The *pex6-1* mutant like *pex5-1* and *pex4-1* was also isolated as an IBA response mutant in Arabidopsis [32]. It shows only a slight import defect and interestingly has reduced levels of PEX5, a phenomenon which is also seen in human and *Pichia pex6* mutants [33, 34]. In contrast, both the *pex4-1* mutant and the *apm4/pex12* mutant have increased levels of PEX5 [22, 29]. *pex1i* also shows classic peroxisome deficiency phenotypes (sucrose dependence, 2,4-DB resistance) and a defect in the import of GFP-PTS1 [28].

Peroxisome proteins show increased turnover under conditions that elevate reactive oxygen species and the *pex4-1/pex22-1* and the *pex6-1* mutants all show stabilization of ICL in Arabidopsis [35]. It is interesting that the *ted3* mutant of PEX2 also results in ICL stabilization [26]. Although there is no direct evidence for peroxisomal ubiqutination in plants, the observation that AtPEX4 and AtPEX22 can complement the corresponding yeast mutants and the role of the E2, AAA and putative E3 proteins in peroxisome associated protein degradation in plants suggests the functioning of an ERAD-type pathway for disposal of damaged matrix proteins [35] (Figure 3B). Other proteases also exist within peroxisomes. DEG15 is required for processing PTS2 proteins, but knockouts do not show an import defect suggesting processing is not required for import [36]. However the peroxisomal targeted LON2 protease does show IBA resistance and a defect in PTS2 import and processing in older seedlings [36]

Import of membrane proteins

Much less is known about import of peroxisome membrane proteins. In mammals and yeast the interplay of PEX3, a membrane protein which is sorted from the ER to peroxisomes, and PEX19, a receptor/chaperone, assembles the peroxisome membrane. PEX16 which is absent in *S. cerevisiae* also plays an important, if enigmatic, role in membrane protein biogenesis. Potential homologues of all these proteins are present in the Arabidopsis genome. PEX3 (two isoforms) and PEX16 have both been shown to sort to peroxisomes [37-39]. No mutants in any of these genes have been isolated by the 2,4-DB or IBA screens. In the case of PEX3 and PEX19 this may be due to the presence of duplicated genes which could be redundant. The *sse1* (*pex16*) mutant was isolated as a mutant which gave a shrunken seed phenotype due to impaired fatty acid synthesis, leading to reduced deposition of storage oil during seed development. However this mutant does show a reduction in PTS1 import [39]. Over-expression of SSE1 causes formation of partially fused peroxisomal aggregates [39] and *pex16i* plants show greatly enlarged peroxisomes which import GFP-PTS1 and appear to contain vesicular inclusions [28]. This is a very different

phenotype from mammalian *pex16* mutants which completely lack detectable peroxisomes. RNAi down regulation of the two PEX19 genes also gave slightly enlarged peroxisomes that contained GFP and *pex3i* contained more tubular peroxisomes [28], again very different phenotypes for those reported from other organisms. Arabidopsis PEX19 has been studied biochemically [40] and shown to bind PEX10. PEX10-YFP is mislocalized in *pex19* RNAi mutants (Brown, unpublished) confirming a role in membrane protein sorting *in vivo*.

Studies on the sorting of individual membrane proteins has provided evidence for two routes. Some proteins such as PMP22 [41], PEX10 and PEX2 [42] are sorted directly to peroxisomes from the cytosol whereas there is good evidence that the peroxisomal isoform of ascorbate peroxidase (APX) is trafficked to peroxisomes via the ER [43]. Surprisingly AtPEX3 is reported to insert directly into peroxisomes [37] in contrast to the situation in yeast, although detecting transient intermediates can be difficult. PEX16 has been reported to traffic to peroxisomes via the ER [44] as it does in mammalian cells [45]. The connection between peroxisomes and the endomembrane system has been a controversial one for many years. However, the most recent evidence favors a eukaryotic origin for peroxisomes and the formation of a pre peroxisome vesicle from the ER. This acquires the membrane bound import machinery (maybe much of it post-translationally) and the capacity to import matrix proteins, and can then divide and segregate to daughter cells (see accompanying article by Hu). If vesicles bring membrane lipids and some proteins from the ER, then a return route most likely operates to return the sorting machinery. As of yet there is little known about the sorting machinery. However viruses may hold the key. The tomato bushy stunt virus (TBSV) protein p33 is targeted to peroxisomes from the cytosol and causes the evagination of the peroxisome membrane. Subsequently p33 and resident peroxisomal membrane proteins are detected in the ER and this is blocked by the expression of a dominant *arf1* mutant [46]. Thus it is an intriguing possibility that TBSV hijacks a yet-to-be characterized endogenous trafficking pathway from the peroxisome to the ER.

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Figure legends

Figure 1. Peroxisomes in leaf epidermal cells.

Peroxisomes labelled with PEX10-eYFP in a stable Arabidopsis line **(A-D)**. Stills taken from the Supplemental Movie 1 highlight the motility of peroxisomes in leaf epidermal cells and the extension of tubular emanations, peroxules (arrowhead). Transient expression of eYFP-PEX11, a peroxisomal membrane protein, and CFP-SKL, a peroxisomal matrix marker, in tobacco leaf epidermal cells **(E-G)**. eYFP-PEX11 surrounds the matrix marker. The void in the centre of the peroxisome is probably due to the presence of a catalase crystal lattice (arrow). Scale bar is 2μm.

Figure 2. Over-view of peroxisomal fatty acid β-oxidation in *Arabidopsis thaliana*

Free fatty acids (or possibly fatty acyl-CoAs) are imported into peroxisomes via the ABC transporter COMATOSE (CTS). Long-chain acyl CoA synthetases (LACS) activate the fatty acids to acyl-CoAs, which then enter the core β-oxidation reactions: acyl-CoA oxidase (ACX), multifunctional protein hydratase (MFP hydratase)/multifunctional protein dehydrogenase (MFP dehydrogenase) and 3-ketoacyl-CoA thiolase (KAT). The end-products are acetyl-CoA (which can be converted to succinate via the glyoxylate cycle to produce carbon skeletons and energy for germinating seedlings) and acyl-CoA shortened by two carbon atoms (which can go through further rounds of β-oxidation). β-oxidation produces toxic H_2O_2, which is broken down mainly by the action of catalase (CAT) and peroxisomal malate dehydrogenase (MDH) is the major enzyme involved in regenerating NAD^+ from NADH for the continued operation of β-oxidation. The isoforms of the various enzymes involved in the pathway are listed on the left along which their respective targeting signal (N/C = noncanonical). Enzymes shaded in grey are not thought to be required for fatty acid β-oxidation in germinating seedlings.

Figure 3: Over-view of components involved in the peroxisomal matrix protein import pathway and ER Associated protein Degradation (ERAD) in *S. cerevisiae*.

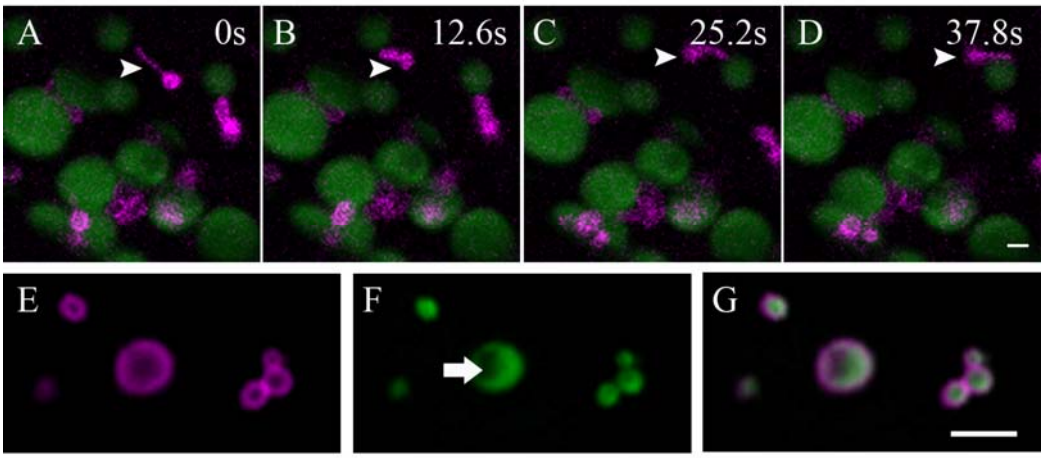
(A) Peroxisomal import machinery. Proteins containing a PTS1 sequence bind to the cytosolic receptor Pex5p and proteins containing a PTS2 sequence bind to the cytosolic receptor Pex7p, which also interacts with co-receptors (PEX5 in mammals and plants, Pex18p/Pex21p in *s. cerevisiae*, Pex20p in other fungi). Interaction with the relevant receptor(s) targets the protein to the docking complex located in the peroxisomal membrane (shown in red, Pex17p is only present in yeast however) and receptor and cargo translocate into the peroxisome lumen where the cargo is then released. The PTS2 sequence is cleaved off via the action of peroxisomal proteases to give the mature protein, but in PTS1-containing proteins the sequence remains. In yeast the docking complex has been shown to associate with the RING complex (blue) via Pex8p, but no homologues of this protein have been identified in mammals and plants. The RING complex consists of the RING-finger E3 ubiquitin ligases Pex2p, Pex10p and Pex12p, all of which are required for Pex5p release from the peroxisomal membrane. Pex10p brings the E2 UBC enzyme Pex4p into association with the RING complex (which is anchored to the peroxisomal membrane via Pex22p) and Pex5p is monoubiquitinated on a conserved cysteine residue present at its N terminus by Pex4p and Pex12p. Plants have homologues for the RING complex, Pex4p and Pex22p, but mammals lack apparent homologues for Pex4p and Pex22p. The AAA-family ATPases Pex1p and Pex6p are thought to recognize monoubiquitinated Pex5p and facilitate its release form the membrane. In mammals and yeast the AAA peroxins are anchored to the peroxisome membrane by a PMP (Pex15p in yeast and PEX26 in mammals). Pex1p and Pex6p have homologues in plants, but it is not known what anchors them to the membrane. The quality

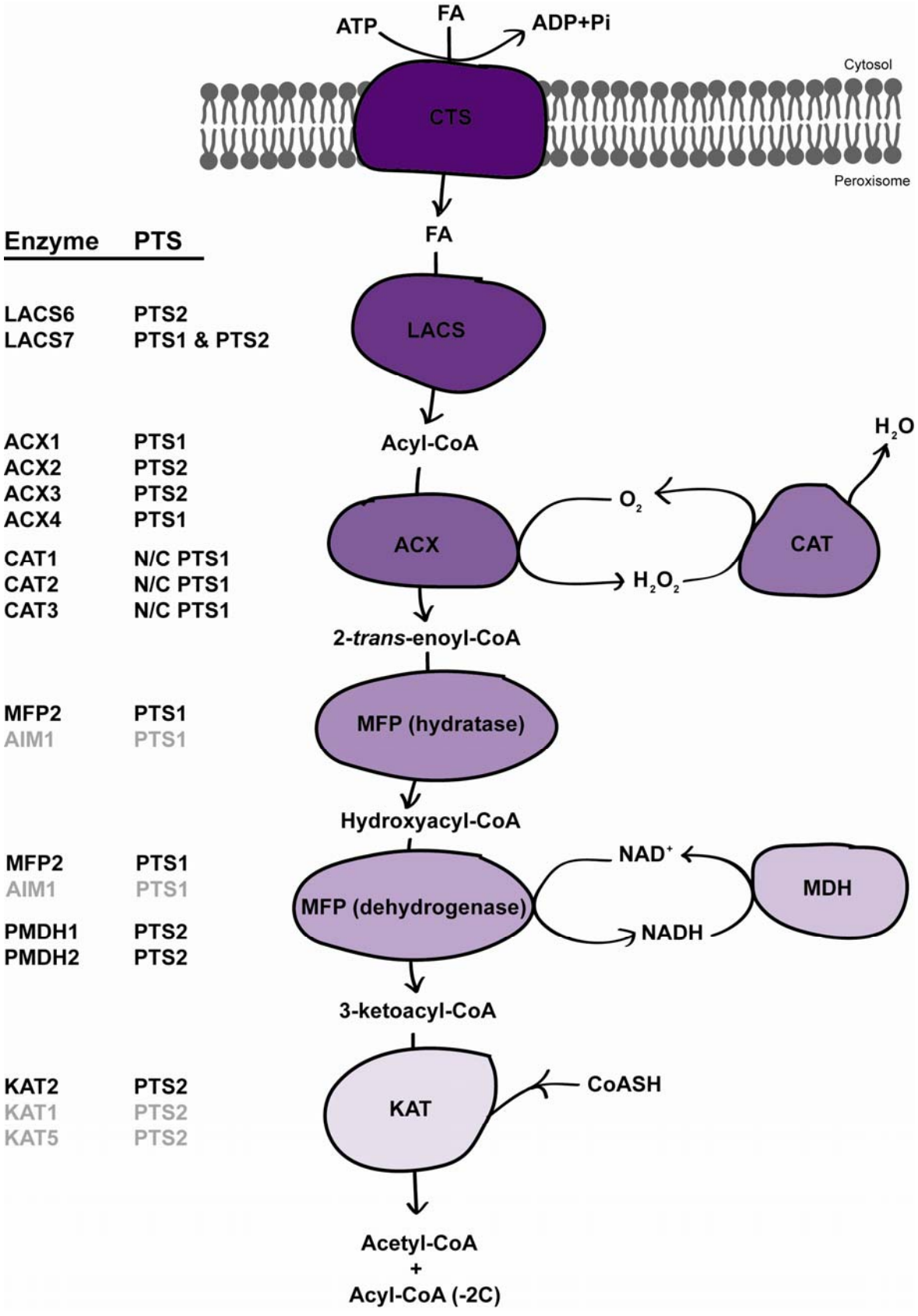
control system termed RADAR (Receptor Accumulation and Degradation in the Absence of Recycling) has also been proposed for the removal of non-functional Pex5p from the peroxisomal membrane. In yeast Pex5p has been shown to be polyubiquitinated by the E2 Ubc4p and Pex2p. There are eight homologues of Ubc4p in plants [47] and in mammals the Ubc4p homologue UBCH5a/b/c has been shown to be required for not polyubiquitination of Pex5p, but monoubiquitination of the conserved cysteine. Polyubiquitinated Pex5p may again be recognized by the AAA peroxins and directed to the proteasome for degradation. Very little is known about the molecular components of Pex7p recycling, although in addition to Pex5p, the co-receptors Pex18p and Pex20p have been shown to be ubiquitinated.

(B) ERAD in yeast. Proteins which have misfolded domains (stars) and are accumulating in the lumen or membrane of the ER can transported into the cytosol and degraded by the proteasome. This process is called ERAD and in yeast proteins misfolded in the lumen are degraded by the ERAD-L pathway, and membrane proteins misfolded in the membrane spanning domain or the cytosolic domain are degraded via the ERAD-M and ERAD-C pathways respectively (reviewed in [48]). There are distinct ubiquitin-ligase complexes involved in the ERAD-L, -M and –C pathways and it has been suggested that these share parallels with the RADAR pathway in peroxisomes. The ERAD pathways require an E3 ligase (Hrd1p or Doa10p, both integral membrane proteins like Pex2/10/12p), an E2 enzyme (Ubc7p, which interacts with Doa10p and is anchored via an integral membrane protein, Cue1p – similar to Pex4p and Pex22p, not shown in this diagram for simplicity) and an AAA-family ATPase (Cdc48p, which interacts with the membrane proteins Ubx2p, Der1p and Usa1p, like Pex6p and Pex15p/PEX26). Additionally the Hrd1p co-factor Hrd3p contains a tetratricopeptide repeat (TPR) motif, which are also present in Pex5p. Yos9p is a lectin-like protein which interacts with Hrd3p and recruits proteins destined for degradation to the Hrd1p/Hrd3p complex. There is no similar component to this in the RADAR pathway.

Supplementary movie 1.

Arabidopsis leaf epidermal cells with chloroplasts in green (autofluorescence detection) and peroxisomes are labelled with PEX10-eYFP.


A
0s
B
12.6s
C
25.2s
D
37.8s
E
F
G


ATP
FA
ADP+Pi
Cytosol
CTS
Peroxisome
Enzyme PTS
FA
LACS6 PTS2
LACS7 PTS1 & PTS2
LACS
Acyl-CoA
ACX1 PTS1
ACX2 PTS2
ACX3 PTS2
ACX4 PTS1
CAT1 N/C PTS1
CAT2 N/C PTS1
CAT3 N/C PTS1
ACX
O2
H2O2
CAT
H2O
2-trans-enoyl-CoA
MFP2 PTS1
AIM1 PTS1
MFP (hydratase)
Hydroxyacyl-CoA
MFP2 PTS1
AIM1 PTS1
PMDH1 PTS2
PMDH2 PTS2
MFP (dehydrogenase)
NAD+
NADH
MDH
3-ketoacyl-CoA
KAT2 PTS2
KAT1 PTS2
KAT5 PTS2
KAT
CoASH
Acetyl-CoA
+
Acyl-CoA (-2C)

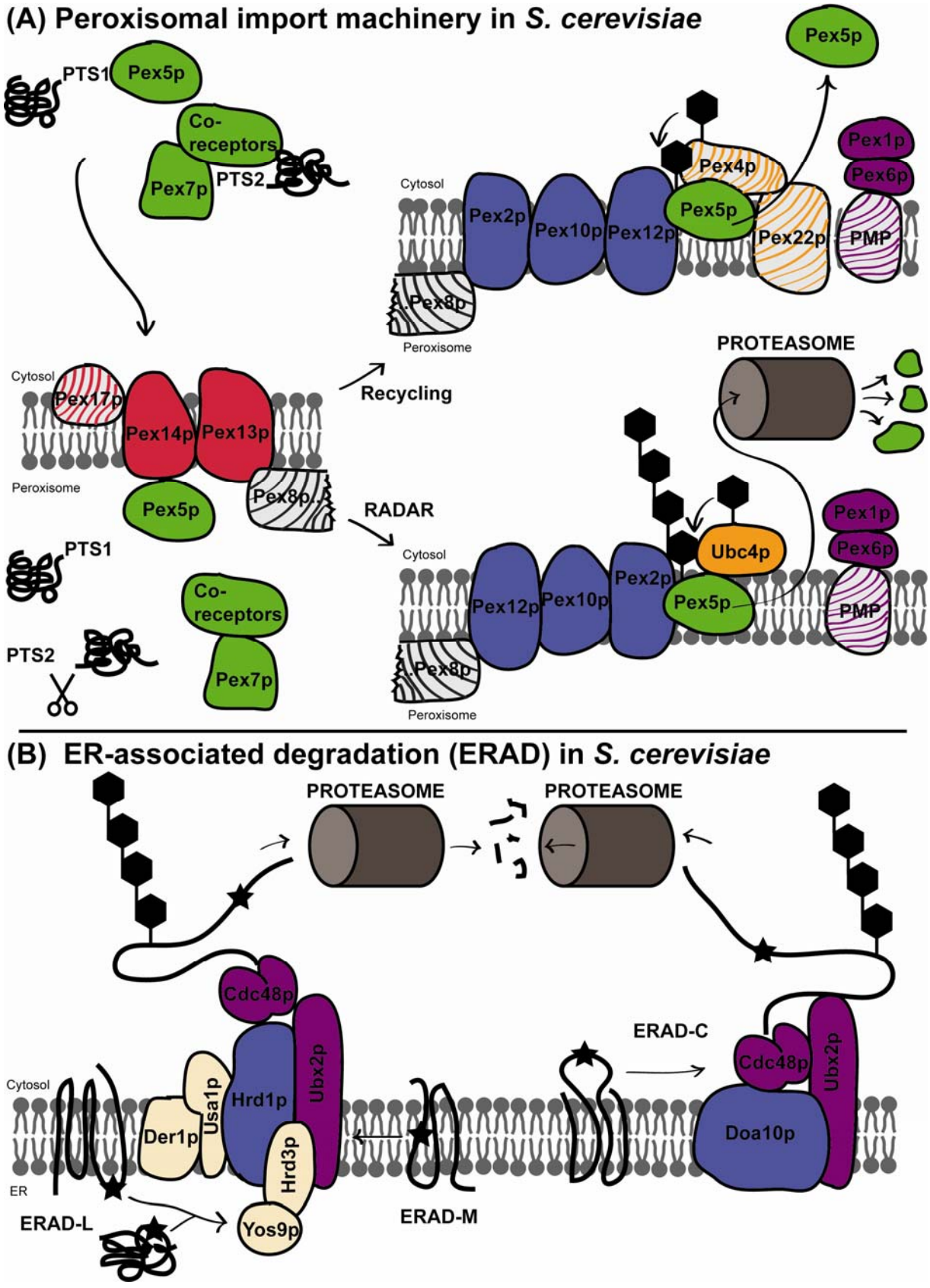

(A) Peroxisomal import machinery in S. cerevisiae
PTS1
Pex5p
Co-receptors
Pex7p
PTS2
Cytosol
Pex2p
Pex10p
Pex12p
Pex4p
Pex5p
Pex22p
Pex1p
Pex6p
PMP
Pex8p
Peroxisome
PROTEASOME
Recycling
Cytosol
Pex17p
Pex14p
Pex13p
Peroxisome
Pex5p
Pex8p
RADAR
PTS1
PTS2
Co-receptors
Pex7p
Cytosol
Ubc4p
Pex1p
Pex6p
Pex2p
Pex12p
Pex10p
Pex5p
PMP
Pex8p
Peroxisome
(B) ER-associated degradation (ERAD) in S. cerevisiae
PROTEASOME
PROTEASOME
Cdc48p
Ubx2p
ERAD-C
Cytosol
Usa1p
Hrd1p
Der1p
Hrd3p
Cdc48p
Ubx2p
Doa10p
ER
ERAD-L
Yos9p
ERAD-M

Table 1. Peroxisome biogenesis mutants in plants.

Gene Name	Mutant	Developmental phenotype	Cellular phenotype	Proposed protein function
PEX1	*pex1i [28]*	Sucrose dependent, 2,4-DB resistant.	PTS1 import defect.	AAA ATPase.
PEX2	*pex2* (T-DNA insertion) [26] *ted3* V275M [26] *pex2i [28]*	Embryo lethal. Gain of function suppressor of *det1-1*. 2,4-DB resistant, sucrose dependent.	 PTS1 import defect.	RING finger protein, possible E3 ubiquitin ligase.
PEX3-1 *PEX3-2*	*pex3-1i* [28] *pex3-2i*	Not sucrose dependent. 2,4-DB sensitive.	No PTS1 or PTS2 import defect, double knock down has elongated, tubular peroxisomes.	Receptor for membrane protein import.
PEX4	*pex4-1* P123L [29] *pex4i* [28]	IBA resistant, sucrose dependent, adult plants normal.	Mild PTS2 import defect PTS1 import defect.	Possible E2 ubiquitin ligase.
PEX5	*pex5-1* S318L [18] *pex5i* RNAi [17]	IBA resistant. 2,4-DB resistant, adult plants small & pale.	PTS2 import defective. PTS1 and 2 import defective.	PTS1 import receptor and co-receptor for PEX7.
PEX6	*pex6-1* RQ [32] *pex6i* [28]	Seedlings IBA resistant, sucrose dependent. Adult plants small, pale, reduced seed set.	Fewer, larger peroxisomes. Reduced PEX5 levels. Over expression of PEX5 partially rescues. PTS1 import defect.	AAA ATPase required for removal of proteins from peroxisomes.
PEX7	*pex7-1*(T-DNA in 5'UTR) [18] *pex7i* RNAi [17]	2,4DB and IBA resistant, not sucrose dependent. IBA resistant sucrose dependent, adult plants normal	PTS2 import defective. PTS2 import defect.	Import receptor for PTS2 proteins.
PEX10	*pex10* Ds element at +599 (Exon 4) [24, 27]	Embryo lethal.		RING finger protein, possible E3 ubiquitin

				ligase.
PEX10	*pex10i*[28]	Very small seedlings, sucrose dependent. Older plants pale with variegated leaves.	PTS1 and 2 import defective.	
PEX12	*pex12* (T-DNA) [25] *apm4* R170K [22] *pex12i*[25]	Embryo lethal. 2,4-DB resistant, reduced germination and establishment.	PTS1 and PTS2 import defect.	RING finger protein, possible E3 ubiquitin ligase.
PEX13	*apm2* Q313stop [22]	Not sucrose dependent, smaller seedling and adult plants. Slight 2,4-DB resistance.	Decreased PTS1&2 import.	Membrane docking partner for PEX5 and 7.
	amc (T-DNA insertion) [23]	Defective male-female gametophyte recognition.	PTS1 import defect.	
	pex13i [28]	Sucrose dependent and 2,4-DB resistant	PTS1 import defect.	
PEX14	*ped2* Q254 stop [20]	2,4-DB resistant, sucrose dependent, compromised photorespiration.	PTS1 and 2 import defective.	Membrane docking partner for PEX5 and 7.
PEX16	*sse1* (T-DNA insertion) [39]	Produce shrunken seed due to lipid deposition defect.	Peroxisomes not detectable.	?
	pex16i [28]	2,4-DB resistant, not sucrose dependent.	Large peroxisomes containing vesicles.	
PEX19-1 *PEX19-2*	*Pex19-1i* [28] *Pex19-2i*	Not sucrose dependent or 2,4-DB resistant.	Larger peroxisomes.	Chaperone/receptor for membrane proteins.
PEX22	*pex22-1* (T-DNA insertion 5' to gene) [29]	Very mild but enhances phenotype of *pex4-1* mutant.	*pex4-1pex22-1* double mutant has delayed turnover of ICL.	Membrane anchor for PEX4.
LON2	*lon2-1, lon2-2* [36]	Weak sucrose dependence, IBA resistant.	Defect in PTS2 processing. PTS2 import defect in older seedlings.	Protease.