Interdependence of the Peroxisome-targeting Receptors in Arabidopsis thaliana: PEX7 Facilitates PEX5 Accumulation and Import of PTS1 Cargo into Peroxisomes

Naxhiely Martínez Ramón and Bonnie Bartel

Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77005

Submitted August 10, 2009; Revised January 19, 2010; Accepted January 21, 2010

Address correspondence to: Bonnie Bartel (bartel@rice.edu).

Abbreviations used: IBA, indole-3-butyric acid; PEX, peroxin; PTS, peroxisome-targeting signal.

Peroxisomes compartmentalize certain metabolic reactions critical to plant and animal development. The import of proteins from the cytosol into the organelle matrix depends on more than a dozen peroxins (PEX) proteins, with PEX5 and PEX7 serving as receptors that shuttle proteins bearing one of two peroxisome-targeting signals (PTSs) into the organelle. PEX5 is the PTS1 receptor; PEX7 is the PTS2 receptor. In plants and mammals, PEX7 depends on PEX5 binding to deliver PTS2 cargo into the peroxisome. In this study, we characterized a pex7 missense mutation, pex7-2, that disrupts both PEX7 cargo binding and PEX7-PEX5 interactions in yeast, as well as PEX7 protein accumulation in plants. We examined localization of peroxisomally targeted green fluorescent protein derivatives in light-grown pex7 mutants and observed not only the expected defects in PTS2 protein import but also defects in PTS1 import. These PTS1 import defects were accompanied by reduced PEX5 accumulation in light-grown pex7 seedlings. Our data suggest that PEX5 and PEX7 import depend on the PTS2 receptor PEX7 in Arabidopsis and that the environment may influence this dependence. These data advance our understanding of the biogenesis of these essential organelles and provide a possible rationale for the retention of the PTS2 pathway in some organisms.

INTRODUCTION

Because peroxisomes lack DNA, all necessary proteins are encoded in the nucleus and imported posttranslationally from the cytosol. There are two well-characterized signals that can direct proteins to enter the peroxisome matrix. The peroxisome-targeting signal (PTS) 1 consists of tripeptide variants at the extreme C terminus, and the PTS2 is a nonapeptide within the first ~30 amino acids of the protein (reviewed in Brown and Baker, 2008). PTS2 proteins comprise approximately a quarter of plant peroxisomal proteins (Kamada et al., 2003; Reumann et al., 2004), including several enzymes necessary for fatty acid β-oxidation that are required for normal seedling development (Hayashi et al., 1998; Hooks et al., 1999; Eastmond et al., 2000; Adham et al., 2005; Pracharoenwattana et al., 2005, 2007).

Two PTS receptors bind and escort cargo proteins from the cytosol to the peroxisome for import: peroxin (PEX) 5 and PEX7, which recognize PTS1- (Brocard et al., 1994; Dödt et al., 1995; Fransen et al., 1995) and PTS2-containing proteins (Rehling et al., 1996), respectively. Receptors and cargo are translocated into the peroxisome, the cargo dissociates, and the receptors are recycled back to the cytosol where they can undergo further rounds of import (Dammam and Subramani, 2001; Nair et al., 2004; Brown and Baker, 2008). In plants and vertebrates, PTS2 proteins such as 3-ketoacyl-CoA thiolase (thiolase) can be processed into a mature form by removal of the N-terminal PTS2 recognition sequence upon entrance into the peroxisome (Brown and Baker, 2008).

Dissecting interactions among peroxins from different species has revealed differences in PEX5 and PEX7 docking with the PEX13 and PEX14 membrane peroxins (Brown and Baker, 2008). Some of the receptor docking differences between mammals and yeast reflect the existence in mammals of long (L) and short (S) isoforms of PEX5 that result from alternative splicing; both isoforms can bind the PTS1, but only PEX5L binds PEX7 and functions in PTS2 import (Braverman et al., 1998; Otera et al., 1998). Arabidopsis seems to only encode a PEX5L isoform, which is required for both PTS1 and PTS2 import (Hayashi et al., 2005; Woodward and Bartel, 2005). Like humans, rice has two PEX5 splice isoforms, and only the longer isoform binds PEX7 (Lee et al., 2006). Fungal PEX5 resembles PEX5S; PEX7 is bound by other fungal peroxins that serve the role in PTS2 import that is served by PEX5L in other organisms (Brown and Baker, 2008). Interestingly, the nematode Caenorhabditis elegans lacks PEX7 and the corresponding PTS2 import pathway; proteins that contain a PTS2 in other organisms are PTS1-targeted in C. elegans (Gurvitz et al., 2000; Motley et al., 2000).

PEX7 and PTS2 import are dependent on the PTS1 receptor PEX5 in many multicellular organisms. In this study, we provide evidence that the converse is also true in plants. We have characterized a new Arabidopsis pex7 allele, pex7-2, that displays defects not only in PEX7 accumulation and PTS2 import but also in PEX5 accumulation and PTS1 import. Our results support the hypothesis that PEX5 and PEX7 are interdependent in plants.
MATERIALS AND METHODS

Plant Material and Growth Conditions

All Arabidopsis thaliana plants were in the Columbia (Col-0) accession. pex7-2 was isolated as an indole-3-butyric acid (IBA)-response mutant from progeny of ethylmethanesulfonate-mutagenized Col-0 seeds (Lehle Seeds, Round Rock, TX) as described previously (Adham et al., 2005; Zolman et al., 2008) and was backcrossed once before phenotypic analyses. The pex7-7 mutant contains a T-DNA inserted 95 base pairs upstream of the start codon in the PEX7 5′-untranslated region (UTR) and has been characterized previously (Chen and Bartel, 2005), pex7-10 contains a T-DNA inserted in PEX5 exon 5 (Zolman et al., 2005), and pex5-1 is a missense allele (Zolman et al., 2000).

For phenotypic assays, seeds were surface sterilized, stratified as indicated, and germinated in 9-cm Petri dishes (Lawrence Livermore National Laboratory, Livermore, CA) as described previously (Adham et al., 2005). Seedlings were grown at 22°C in the dark or under continuous white or yellow-filtered light (Stinson-poulos and Hangarter, 1990), as indicated. Dark-grown plants were grown horizontally for 1 d under white light then transferred to the dark and incubated on vertically oriented plates; light-grown plants were incubated on horizontally oriented plates.

Map-Based Cloning

DNA was isolated from IBA-resistant plants selected from F2 progeny of pex7-2 crossed to Ler-0. Mapping with polymerase chain reaction (PCR)-based molecular markers (Supplemental Table 1) localized the lesion to chromosome 1 between F6N18 and F28N24. The At1g229260 gene was PCR amplified from genomic DNA prepared from the mutant and sequenced (Lynx Technologies, Rock, TX). The PEX7 cDNA was driven by the cauliflower mosaic virus 35S promoter by subcloning the Sall/NotI insert of pc4-PEX7 (see below) into Xhol/NotI-cut 35S/pBARN (LeClerc and Bartel, 2001) to give 35S-PEX7a, which was transformed into pex7-2 by using the floral dip method (Clough and Bent, 1998). T1 plants were selected on 7.5 μg/ml glucofructose amonium, and homozygous plants were selected from subsequent generations by analyzing the pattern of seedling glucofructose ammonium resistance.

Immunoblot Analysis

Seedling protein was extracted by grinding tissue on dry ice and then mixing with an equal volume of NuPAGE 2x loading buffer (Invitrogen, Carlsbad, CA). After centrifugation, supernatants were loaded onto NuPAGE 10% Bis-Tris gels (Invitrogen) next to broad-range Malters (Santa Cruz Biotechnology, Santa Cruz, CA). After electro transfer, membranes were incubated with mouse anti-HSC70 (1:5000, SPA-817; Assay Designs, Clifton, NJ) or rabbit anti-PEX5 (1:1000, sc-2031; Santa Cruz Biotechnology). Horseradish peroxidase was visualized using LumiGLO (Cell Signaling Technology, Danvers, MA) according to the manufacturer’s instructions.

RESULTS

Isolation of an IBA-Response Mutant Defective in PEX7

Because peroxisomes are the site of fatty acid β-oxidation and metabolism of the auxin precursor IBA, sucrose dependence and IBA resistance can be used to assess peroxisome function in Arabidopsis seedlings (Zolman et al., 2000). To identify peroxisome defects, we screened for mutants with IBA-resistant root elongation and used recombinant mapping to localize the lesion in one such mutant to a region on chromosome 1 (Figure 1A) containing PEX7 (At1g229260), which encodes the Arabidopsis PTS2 receptor. Because the previously characterized pex7-1 mutant, which accumulates reduced PEX7 mRNA levels due to a T-DNA insertion in the PEX7 5′-UTR, also displays reduced IBA response (Woodward and Bartel, 2005), we sequenced the PEX7 coding region from mutant DNA. We found a C-to-T base change at position 371 (where the A of the ATG start codon is position 1) that results in a Thr124-to-Ile amino acid change in the second WD-40 repeat of PEX7 (Figure 1A). Although the PEX7 structure has not been solved from any organism, WD-40 proteins typically fold into β-propeller structures, with each propeller blade comprised of four β-strands. The identified mutation is predicted to fall near the C-terminal end of the second β-strand in the second propeller blade of PEX7. We named this new allele pex7-2.

To determine whether the IBA-resistant root elongation of pex7-2 was caused by the mutation in the PEX7 gene, we conducted a complementation test with pex7-1. We found that pex7-2 was recessive and that pex7-2 failed to complement the pex7-1 resistance to IBA (Figure 1B). Moreover, we could fully restore pex7-2 mutant defects by expressing a wild-type PEX7 cDNA from the cauliflower mosaic virus 3Ss promoter in pex7-2 (Figure 1C and D), confirming that the identified PEX7 lesion was responsible for the pex7-2 mutant phenotypes.
Peroxisome-defective Physiological Phenotypes in pex7-2
We used physiological assays that require peroxisomal metabolism to compare the extent of peroxisomal deficiency in the pex7-2 mutant to pex7-1 (Woodward and Bartel, 2005) and two mutants defective in the PTS1 receptor PEX5, the

Figure 1. Positional cloning of the gene defective in pex7-2. (A) Recombination mapping localized the defect between F28N24 and F6N18. This interval includes the PEX7 gene (At1g29260), which encodes the PTS2 receptor. The pex7-2 C-to-T mutation causes a Thr-to-Ile missense mutation in the second WD-40 repeat; the triangle shows the position of the pex7-1 T-DNA insertion in the 5'-UTR (Woodward and Bartel, 2005). (B) pex7-2 fails to complement pex7-1. Four 8-d-old seedlings representing the range of root lengths after growth under yellow-filtered light on medium without or with 10 μM IBA are shown. Col-0 plants were used as wild type (PEX7/PEX7), and the mean percentage of root length on IBA versus unsupplemented medium is shown; n = 10. Bar, 1 cm. (C) 35S-PEX7a rescues pex7-2 sucrose dependence. Seedlings were grown under white light for 1 d and transferred to the dark for five additional days on medium with or without 0.5% sucrose. Bars show mean hypocotyl lengths +/- SE; n = 9. (D) 35S-PEX7a restores pex7-2 IBA responsiveness. Bars show mean root lengths (+SE; n = 10) of 8-d-old seedlings grown under yellow-filtered light on 10 μM IBA or medium containing no hormone.

pex5-10 T-DNA insertion allele (Zolman and Bartel, 2004). pex7-2
displayed resistance to inhibition of root elongation by IBA in plants similar to pex5-10 and pex7-2, responding to IBA at higher concentrations, whereas pex5-10 seemed unresponsive to IBA in this assay (Figure 2A and Supplemental Figure 1A). pex7-2 was more resistant to the promotion of lateral roots by IBA than pex7-1, but like other pex mutants, still responded robustly to the promotion of lateral roots by the synthetic auxin 1-naphthaleneacetic acid (NAA) (Figure 2B), implicating conversion of the IBA protoauxin to active indole-3-acetic acid rather than the ability to form lateral roots as the pex7-2 mutant defect.

In addition to IBA response defects, peroxisome-defective mutants often fail to efficiently β-oxidize seed storage fatty acids following germination, resulting in developmental arrest or delay that can be restored by provision of sucrose (Hayashi et al., 1998; Zolman et al., 2000). We found that pex7-2, unlike pex7-1, was partially sucrose dependent in the dark, similar to pex5-1 but less severe than pex5-10 (Figure 2C and Supplemental Figure 1B). When grown in the light, however, pex7-2 was severely sucrose dependent, whereas pex5-1 and pex7-1 resembled wild type (Figure 2C and Supplemental Figure 1C). When grown on sucrose in the light, pex7-2 seedlings were smaller than pex7-1 and pex5-1, which resembled wild type, but not as small as pex5-10 (Figure 3A). Together, the pex7-2 phenotypes suggested a more severe defect in peroxisome function, and therefore PEX7 function, in pex7-2 than in pex7-1.

Enhanced Peroxisome-defective Phenotypes in pex7 pex5 Double Mutants

The phenotypically weak pex7-1 mutation dramatically enhances pex5-1 phenotypes, consistent with the finding that both single mutants confer partial defects in PTS2 import (Woodward and Bartel, 2005). In spite of these defects, the pex5-1 pex7-1 double mutant survives to produce some viable seed (Woodward and Bartel, 2005). In contrast, we were unable to recover viable pex5-1 pex7-2 or pex5-10 pex7-2 double mutants. For example, we analyzed the progeny of pex5-1/pex5-1 pex7-2/PEX7 individuals and found 69% (18/26) pex5-1/pex5-1 pex7-2/PEX7 plants and 31% (8/26) pex5-1/pex5-1 PEX7/pex7-2 plants, suggesting that the pex5-1 pex7-2 combination is lethal. Similarly, in the progeny of pex5-10/PEX5 pex7-2/PEX7 plants, we observed 62% (8/13) pex5-10/PEX5 pex7-2/pex7-2 plants and 38% (5/13) PEX5/PEX5 pex7-2/pex7-2 plants, suggesting that pex5-10 pex7-2 is also lethal. Altogether, we analyzed 343 progeny of pex5-1 × pex7-2 crosses and 91 progeny of pex5-10 × pex7-2 crosses without recovering either double mutant. We analyzed developing seeds in pex5-1/pex5-1 pex7-2/PEX7 and pex5-10/PEX5 pex7-2/PEX7 siliques and found shriveled seeds diagnostic of embryonic lethality (Figure 3C). At maturity, these plants generated ~10% shrunk seeds (Figure 3D). We concluded that combining pex7-2 with either pex5-1 or pex5-10 confers embryonic lethality.

In contrast to the inviability of the pex5 pex7-2 double mutants, we were able to isolate the pex5-10 pex7-1 double mutant. The pex5-10 pex7-1 mutant resembled pex5-10; it did not germinate without sucrose (Figure 2C) and displayed similar IBA resistance as pex5-10 in root elongation (Figure 2A) and lateral root initiation (Figure 2B) assays. The general growth and development of pex5-10 pex7-1 was also similar to that of pex5-10; both mutants were slow to develop even when provided with sucrose in the light or the dark (Figures 2C and 3A) but eventually produced fertile adult plants (Figure 3B). The viability of pex5 pex7-1 double mutants and the inviability of pex5 pex7-2 double mutants are consistent with our phenotypic analyses of the relative defects in the pex7-1 and pex7-2 single mutants, which also suggest that pex7-2 more completely blocks PEX7 function than pex7-1.

pex7-2 Disrupts Binding to PEX5 and PTS2-Cargo in Yeast Two-Hybrid Assays

In plants and mammals, PEX7 must bind both PTS2 cargo and the PTS1 receptor PEX5 to deliver PTS2 cargo to peroxisomal membranes.
oxisomes (Braverman et al., 1998; Otera et al., 1998; Nito et al., 2002; Hayashi et al., 2005; Woodward and Bartel, 2005). We used directed yeast two-hybrid assays to determine whether one or both of these functions was altered in pex7-2. As demonstrated previously (Nito et al., 2002), we found that Arabidopsis PEX7 and PEX5 interacted in the yeast two-hybrid assay. In contrast, pex7-2 did not interact with PEX5 in this assay (Figure 4A).

We also compared PEX7 and pex7-2 interactions with the PTS2 protein thiolase (PED1) to determine whether the pex7-2 mutation disrupted cargo binding. As expected, PEX7 interacted with PED1 but not with an N-terminally truncated PED1 version (tPED1) lacking the PTS2 region (Figure 4A). In contrast, pex7-2 paired with PED1 did not grow on selective medium (−His) and plates on which growth requires interaction (+His) are shown after incubation at 30°C for 2 d. (B) Proteins extracted from yeast strains in A were separated using SDS-polyacrylamide gel electrophoresis and immunoblots were probed with the indicated antibodies. Extracts from 8-d-old wild-type Arabidopsis seedlings and the untransformed AH109 yeast were included as controls.

PTS Import Defects in pex7-2 Peroxisomes
We indirectly assessed PTS2 cargo import into pex7 peroxisomes by monitoring removal of the PTS2 signal from thiolase and peroxisomal malate dehydrogenase (PMDH). PTS2 processing occurs after import and is catalyzed by the peroxisomally localized PTS2-processing protease DEG15, a PTS1 protein (Helm et al., 2007; Schuhmann et al., 2008). Similar to previous reports of thiolase processing in pex7-1, pex5-10, and pex7-1 pex5-1 (Woodward and Bartel, 2005; Zolman et al., 2005), we found that in light-grown seedlings, thiolase and PMDH were fully processed in wild type, partially processed in the pex7-1, pex7-2, and pex5-1 single mutants, and only minimally processed in pex5-10 and the double mutants (Figure 5A). We found similar thiolase processing defects in dark-grown seedlings (Figure 5C), although pex7-2 defects seemed slightly less severe. Approximately half of the thiolase was processed in dark-grown pex7-2 seedlings (Figure 5C), whereas nearly all thiolase was unprocessed in light-grown pex7-2 seedlings (Figure 5A).

To directly analyze the effects of the pex7-2 mutation on matrix protein import, we crossed pex7-2 to transgenic lines containing peroxisome-targeted GFP and examined the resultant homozygous plants using fluorescence microscopy. Whereas wild-type cotyledon cells efficiently imported PTS2-GFP into punctate structures indicative of peroxisomes, we found that PTS2-GFP displayed the expected punctate peroxisomal localization in pex7-2 seedlings (Figure 6A). This localization confirmed that PTS2 import is impaired but not completely blocked in pex7-2. We also examined a PTS1 reporter and found that GFP-PTS1 displayed the expected punctate peroxiso-
of 7-d-old light-grown seedlings or of seedlings grown in light fluorescence. Bar, 20 μm.

Figure 6. pea7-2 defects in matrix protein import. (A) Cotyledons of 7-d-old light-grown seedlings or of seedlings grown 1 d in light and 4 d in darkness on 0.5% sucrose were analyzed using confocal fluorescence microscopy. PTS2 protein targeting was visualized by imaging fluorescence from a GFP derivative (PTS2-GFP) carrying the N-terminal peroxisome targeting signal from PED1 (Woodward and Bartel, 2005) in epidermal cells (first column) and the underlying mesophyll cells (second column). The mesophyll column shows merged images of GFP (green) and chlorophyll (chl; magenta) fluorescence. The large central vacuole of expanded plant cells consolidates most of the cytosol at the cell margins. Dark-grown cotyledons were briefly stained with propidium iodide, which stains cell walls, before visualization of epidermal cells; the last column shows merged image of GFP (green; third column) and propidium iodide (magenta) fluorescence. Bar, 20 μm. (B) Cotyledons of seedlings grown as described in A were analyzed as described in A. PTS1 protein targeting was visualized by imaging fluorescence from a GFP derivative (GFP-PTS1) carrying a C-terminal PTS1 (Zolman and Bartel, 2004). Bar, 20 μm.

Decreased PEX5 Levels in pea7 Mutants

The cytosolic fluorescence pattern of GFP-PTS1 in light-grown pea7 mutants suggested that PTS1 import, and by extension PEX5, depends on PEX7. To further understand the basis of the pea7 PES1 import defects, we analyzed peroxin levels in mutant seedlings. We found that PEX7 levels were similar to wild type in both pea5 alleles but were reduced in both pea7 alleles (Figure 5, B and D), consistent with the reduced PEX7 mRNA level in pea7-1 (Woodward and Bartel, 2005) and suggesting that the pea7-2 lesion might impair PEX7 stability in seedlings in addition to disrupting PEX5 and cargo interactions (Figure 4A). As reported previously (Woodward and Bartel, 2005; Zolman et al., 2005), pea5-1 and pea5-10 mutants accumulated normal and undetectable levels of full-length PEX5, respectively. A smaller protein that cross-reacts with our PEX5 antibody, which was generated to a peptide corresponding to the PEX5 C terminus (Zolman and Bartel, 2004), was sometimes detected in pea5-10 extracts (Figure 5, B and D). This residual immunoreactivity, along with the small fraction of processed thiolase detected even in pea5-10 plants (Figure 5C; Zolman et al., 2005), is consistent with the possibility that the pea5-10 protein may retain partial function.

Although PEX5 levels were nearly normal in pea7 mutants grown in the dark (Figure 5D), light-grown seedlings of both pea7-1 and pea7-2 accumulated substantially less PEX5 protein than wild type (Figure 5B). This result suggested that the defects in GFP-PTS1 import displayed by the pea7 mutants could be explained by reduced PEX5 accumulation in these mutants and that PEX5 might depend on PEX7 for stability in light-grown seedlings.

DISCUSSION

Import of PTS2 cargo into mammalian and plant peroxisomes requires not only the PTS2 receptor PEX7 but also the PTS1 receptor PEX5 (Braverman et al., 1998; Otera et al., 1998; Hayashi et al., 2005; Woodward and Bartel, 2005; Lee et al., 2006). In this work, we used a new pea7 allele, pea7-2, to demonstrate that the reciprocal is also true: Arabidopsis PEX5 and PTS1 import depend on the PTS2 receptor PEX7.

The pea7-2 mutant displayed typical peroxisome-defective phenotypes such as IBA resistance and sucrose dependence during seedling development (Figure 2). The pea7-2 mutant defects were more severe than the previously characterized pea7-1 and pea5-1 mutants (Zolman et al., 2000; Woodward and Bartel, 2005) but less severe than the pea5-10 T-DNA insertion allele (Figures 2 and 3). We were unable to recover pea5-1 pea7-2 or pea5-10 pea7-2 double mutants (Figure 3), implying that peroxisomal matrix protein import is essential during Arabidopsis embryogenesis. Indeed, mutants defective in multiple isozymes of fatty acid β-oxidation enzymes display embryo lethality (Rylott et al., 2003, 2006), suggesting that one essential embryonic function for peroxisomes is β-oxidation. An essential role for peroxisome matrix protein import in embryogenesis is also suggested by the embryo lethality observed in null alleles of the ring-finger peroxins.
The pex7-2 Thr to Ile missense mutation disrupted interactions with both PTS2 cargo and the PTS1 receptor PEX5 in yeast two-hybrid assays (Figure 4). It remains formally possible that the reduced ability of pex7-2 to bind PEX5 was a secondary effect of reduced cargo binding, as yeast contains several PTS2 proteins that might interact with Arabidopsis PEX7. It seems less likely, however, that the reduced ability of pex7-2 to bind cargo in the yeast two-hybrid assay was a secondary effect of reduced PEX5 binding, as yeast PEX5 does not bind PEX7 (Brown and Baker, 2008).

Interestingly, the pex7-2 mutation resulted in reduced pex7-2 protein accumulation in plants, particularly in light-grown seedlings (Figure 5B). It is possible that the missense mutation impairs receptor folding, thereby rendering it more susceptible to proteolysis in vivo and unable to interact with binding partners. However, Gal4-pex7-2 accumulated normally in yeast (Figure 4B), suggesting that the pex7-2 Thr-to-Ile substitution does not lead to global pex7-2 unfolding. In addition, dark-grown pex7 seedlings accumulated more easily detected amounts of PEX7 protein (Figure 5). Alternatively, pex7-2 may fold correctly but may be subject to proteolysis in vivo because it is not protected by association with PEX5 or with cargo or because it is not efficiently recycled from the peroxisome, similar to what is seen with inefficiently recycled PEX5 (reviewed in Brown and Baker, 2008). Distinguishing among these possibilities will be aided by future studies that elucidate the PEX7 structure and define PEX7 binding interfaces.

We found that along with reduced PEX7 levels, both pex7-2 and pex7-1 displayed reduced PEX5 protein levels when grown in the light (Figure 5), suggesting that PEX5 depends on PEX7 for stability. Moreover, GFP-PTS1 was mislocalized to the cytosol in light-grown pex7 mutants (Figure 6), indicating that the reduced PEX5 levels that we observed in pex7 were accompanied by a reduced efficiency of PTS1 import. Because the pex7-1 mutation results in reduced accumulation of wild-type PEX7 (Woodward and Bartel, 2005), these defects are not a specific consequence of the pex7-2 lesion but likely result from reduced PEX7 function. To our knowledge, a dependence of PTS1 import on the PTS2 receptor has not previously been observed in plants. Interestingly, the possibility that PTS1 import can depend on the PEX7 pathway may not be unique to plants. A recent study in trypanosomes revealed that pex7 RNA interference lines had low PEX5 levels and mislocalized not only PTS2, but also PTS1 proteins (Galland et al., 2007). Moreover, H. polymorpha mutants lacking the PEX7 coreceptor PEX20 also display reduced PEX5 accumulation (Moscicka et al., 2007).

Other pex mutants also impact PEX5 levels. The Arabidopsis pex6 mutant has decreased PEX5 levels (Zolman and Bartel, 2004), consistent with the possibility that Arabidopsis PEX5 is degraded when not properly recycled, as has been demonstrated in nonplant systems (reviewed in Brown and Baker, 2008). Mammalian PEX7 interacts with the PEX2 RING-finger peroxin (Miyata et al., 2009) and Arabidopsis PEX7 interacts with PEX12, another RING-finger peroxin (Singh et al., 2009). In yeast, PEX12 catalyzes the PEX5 monoubiquitination that is essential for PEX5 recycling, whereas PEX2 catalyzes PEX5 polyubiquitination, which leads to PEX5 degradation (Platta et al., 2009). It is possible that the reduced PEX5 levels that we observed in pex7 mutants result from a PEX5 recycling defect, perhaps because PEX7 normally promotes PEX5 association with the recycling components through its association with PEX12.

Our analysis also revealed that the severity of peroxisomal import defects can be impacted by light conditions. The more substantial defects in PEX5 and PEX7 accumulation in the light (Figure 5) were accompanied by more severe pex7-2 sucrose dependence in the light compared with the dark (Figure 2C). Moreover, we observed GFP-PTS1 import defects only in light-grown pex7 seedlings (Figure 6). Other peroxisome-related mutants with light-enhanced sucrose dependence include acx4, a mutant defective in a PTS1-targeted short-chain acyl-CoA oxidase that is sucrose dependent in the light but not in the dark (Adham et al., 2005), and pex6-1, which displays more severe sucrose dependence in the light than in the dark (Zolman and Bartel, 2004). These light-exacerbated phenotypes suggest that particular PEX protein roles might have varied importance in different environmental conditions. It is intriguing that PTS2 proteins are overrepresented among fatty acid β-oxidation enzymes (Kim et al., 2003), which act to metabolize fatty acids stored in seeds immediately after germination, whereas virtually all peroxisomal photoreactivation enzymes are PTS1 proteins. Perhaps the role of PEX7 in promoting PEX5 accumulation becomes more important in the light when there is a reduced demand for PTS2 import.

The majority of peroxisomal matrix proteins are PTS1-containing proteins, and PEX5 has been at the forefront of peroxisome receptor research. Although the fact that C. elegans lacks PEX7 (Gurvitz et al., 2000; Motley et al., 2000) demonstrates that organisms can evolve peroxisomes that function without a PTS2 pathway, the observation that most examined eukaryotes maintain both PEX5 and PEX7 implies that the dual targeting system confers some selective advantage. This study has uncovered an importance for the PTS2 receptor PEX7 in facilitating PTS1 import. Such interdependence among targeting receptors may make it more difficult for one targeting system to be lost. For example, a role for PEX7 in PEX5 function provides a rationale for the continued existence of an apparent PEX7 homologue in Drosophila melanogaster (Motley et al., 2000), even though flies lack readily identifiable PTS2 proteins (Woodward, 2005). Further studies are needed to uncover the molecular mechanisms by which PEX7 promotes PEX5 accumulation and function.

ACKNOWLEDGMENTS

We thank Lucia Strader for microscopy assistance; Arthur Millius and A. Raquel Adham for initial pex7-2 isolation; Neda Nikravan for mapping assistance; Tyler Moss for assistance with PEDI constructs; Andrew Woodward for developing the F28N24 marker and for assistance in generating pex7-1 (GFP-PTS1); Steven Smith for the PMDH2 antibodies; and Sarah Christiansen, Melanie Monroe-Augustus, Sarah Ratzel, Lucia Bartel, and Andrew Woodward for critical comments on the manuscript. This research was supported by the National Science Foundation (MCB-0745122) and the Robert A. Welch Foundation (C-1309). N.M.R. was supported in part by a National Institutes of Health predoctoral fellowship (F31-GM081911) and the Rice Houston Alliance for Graduate Education and the Professoriate Program (NSF HRD-0450363).

REFERENCES

Adham, A. R., Zolman, B. K., Millius, A., and Bartel, B. (2005). Mutations in Arabidopsis acyl-CoA oxidase genes reveal distinct and overlapping roles in β-oxidation. Plant J. 41, 859–874.
Asamizu, E., Nakamura, Y., Sato, S., and Tabata, S. (2000). A large scale analysis of cDNA in Arabidopsis thaliana: generation of 12,028 non-redundant expressed sequence tags from normalized and size-selected cDNA libraries. DNA Res. 7, 175–180.

Ausubel, F., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1999). Current Protocols in Molecular Biology, New York: Greene Publishing Associates and Wiley-Interscience.

Braverman, N., Dodt, G., Gould, S. J., and Valle, D. (1998). An isoform of Pex5p, the human PTS1 receptor, is required for the import of PTS2 proteins into peroxisomes. Hum. Mol. Genet. 7, 1195–1205.

Brocard, C., Kragler, F., Simon, M. M., Schuster, T., and Hartig, A. (1994). The tetratricopeptide repeat-domain of the PAX10 protein of Saccharomyces cerevisiae is essential for binding to the peroxisomal targeting signal -SKL. Biochem. Biophys. Res. Commun. 204, 1016–1022.

Brown, L.-A., and Baker, A. (2008). Shuttles and cycles: transport of proteins into the peroxisome matrix. Mol. Membr. Biol. 25, 363–375.

Clough, S. J., and Bent, A. F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 16, 735–743.

Dammari, V., and Subramani, S. (2001). The human peroxisomal targeting signal receptor, Pex5p, is translocated into the peroxisome matrix and recycled to the cytosol. Cell 105, 187–196.

Dodt, G., Braverman, N., Wong, C., Moser, A., Moser, H. W., Watkins, P., Valle, D., and Gould, S. J. (1995). Mutations in the PTS1 receptor gene, PXR1, define complementation group 2 of the peroxisome biogenesis disorders. Nat. Genet. 9, 115–125.

Eastmond, P. J., Hooks, M. A., Williams, D., Lange, P., Bechtold, N., Sarrobert, C., Nussaume, L., and Graham, I. A. (2000). Promoter trapping of a novel medium-chain acyl-CoA oxidase, which is induced transcriptionally during seed germination. J. Biol. Chem. 275, 34375–34381.

Fan, J., Quan, S., Orth, T., Awai, C., Chory, J., and Hu, J. (2005). The Arabidopsis PEX12 gene is required for peroxisome biogenesis and is essential for developmental. Plant Physiol. 139, 231–239.

Fransen, M., Brees, C., Baumgart, E., Vanhooren, J.C.T., Baes, M., Mannenta, G. P., and Veldhoven, P. P. (1995). Identification and characterization of the putative human peroxisomal C-terminal targeting signal import receptor. J. Biol. Chem. 270, 7731–7736.

Galland, N., Demeure, F., Hanaaert, V., Verplaatse, E., Vertommen, D., Van der Smissen, P., Courtoy, P. J., and Michelos, P. A. (2007). Characterization of the role of the receptors PEXS and PEX7 in the import of proteins into glycosomes of Trypanosoma brucei. Biochim. Biophys. Acta 1773, 521–535.

Gietz, R., and Schiestl, R. (1995). Transforming yeast with DNA. Methods Mol. Cell. Biol. 5, 255–269.

Gurvitz, A., Langer, S., Fiskacek, M., Hamilton, B., Ruis, H., and Hartig, A. (2000). Predicting the function and subcellular location of Caenorhabditis elegans proteins similar to Saccharomyces cerevisiae proteins. Mol. Membr. Biol. 17, 101–109.

Hayashi, M., Yagi, M., Nito, K., Kamada, T., and Nishimura, M. (2002). Direct interaction and determination of binding domains among peroxisomal import factors in Arabidopsis thaliana. Plant Cell Physiol. 43, 535–566.

Nair, D. M., Purdy, P. E., and Lazarov, P. B. (2004). Pex7p translocates in and out of peroxisomes in Saccharomyces cerevisiae. J. Cell Biol. 167, 599–604.

Nito, K., Hayashi, M., and Nishimura, M. (2002). Direct interaction and determination of binding domains among peroxisomal import factors in Arabidopsis thaliana. Plant Cell Physiol. 43, 535–566.

Otera, H., et al. (1998). Peroxisome targeting signal type 1 (PTS1) receptor is involved in import of both PTS1 and PTS2, Studies with PEX5-defective CHO cell mutants. Mol. Cell. Biol. 18, 388–399.

Platta, H. W., Magraoui, E. F., Baumer, B. E., Schiere, D., Girzalsky, W., and Erdmann, R. (2009). Pex2 and Pex12 function as protein-ubiquitin ligases in peroxisomal protein import. Mol. Cell. Biol. 29, 5525–5536.

Pracharoenwattana, I., Cornah, J. E., and Smith, S. M. (2005). Arabidopsis peroxisomal citrate synthase is required for fatty acid respiration and seed germination. Plant Cell 17, 2037–2048.

Pracharoenwattana, I., Cornah, J. E., and Smith, S. M. (2007). Arabidopsis peroxisomal malate dehydrogenase functions in β-oxidation but not in the glyoxylate cycle. Plant J. 50, 381–390.

Rehling, P., Marzioch, M., Niesen, F., Wüthke, E., Veenhuis, M., and Kunau, W. H. (1996). The import receptor for the peroxisomal targeting signal 2 (PTS2) in Saccharomyces cerevisiae is encoded by the PASY gene. EMBO J. 15, 2901–2913.

Reumann, S., Ma, C., Lemke, S., and Babuji, L. (2004). AraPerox. A database of putative Arabidopsis proteins from plant peroxisomes. Plant Physiol. 136, 2587–2598.

Rylott, E. L., Eastmond, P. J., Gilday, A. D., Slocombe, S. P., Larson, T. R., Baker, A., and Graham, I. A. (2006). The Arabidopsis thaliana multifunctional protein gene (MEP2) of peroxisomal beta-oxidation is essential for seedling establishment. Plant J. 45, 901–911.

Rylott, E. L., Rogers, C. A., Gilday, A. D., Edgell, T., Larson, T. R., and Graham, I. A. (2003). Arabidopsis mutants in short- and medium-chain acyl-CoA oxidase activities accumulate acyl-CoAs and reveal that fatty acid β-oxidation is essential for embryo development. J. Biol. Chem. 278, 21370–21377.

Schuhmann, H., Huesgen, P. F., Gietl, C., and Adamska, I. (2008). The DEG15 serine protease cleaves peroxisomal targeting signal 2-containing proteins in Arabidopsis. Plant J. 54, 1847–1856.

Schumann, U., Wanner, G., Veenhuis, M., Schmid, M., and Gietl, C. (2003). AthPEX10, a nuclear gene essential for peroxisome and storage organelle formation during Arabidopsis embryogenesis. Proc. Natl. Acad. Sci. USA 100, 9826–9831.

Singh, T., Hayashi, M., Mano, S., Arai, Y., Goto, S., and Nishimura, M. (2009). Molecular Components Required for the Targeting of PEX10 to Peroxisomes in Arabidopsis thaliana. Plant J. 60, 488–498.

Sparkes, I. A., Brandizzi, F., Slocombe, S. P., El-Shami, M., Hawes, C., and Baker, A. (2003). An Arabidopsis pex10 null mutant is embryo lethal, implicating peroxisomes in an essential role during plant embryogenesis. Plant Physiol. 133, 1809–1819.

Stasinopoulos, T. C., and Hangarter, P. R. (1990). Preventing photochemistry in culture media by long-pass light filters alters growth of cultured tissues. Plant Physiol. 93, 1365–1369.
Woodward, A. W. (2005). Genes, Organelles, and Molecules That Influence Plant Development through Auxin Regulation. Ph.D. Dissertation. Houston, TX: Rice University.

Woodward, A. W., and Bartel, B. (2005). The Arabidopsis peroxisomal targeting signal type 2 receptor PEX7 is necessary for peroxisome function and dependent on PEX5. Mol. Biol. Cell 16, 573–583.

Yamada, K., et al. (2003). Empirical analysis of transcriptional activity in the Arabidopsis genome. Science 302, 842–846.

Zolman, B. K., and Bartel, B. (2004). An Arabidopsis indole-3-butyric acid-response mutant defective in PEROXIN6, an apparent ATPase implicated in peroxisomal function. Proc. Natl. Acad. Sci. USA 101, 1786–1791.

Zolman, B. K., Martinez, N., Millius, A., Adham, A. R., and Bartel, B. (2008). Identification and characterization of Arabidopsis indole-3-butyric acid response mutants defective in novel peroxisomal enzymes. Genetics 180, 237–251.

Zolman, B. K., Monroe-Augustus, M., Silva, I. D., and Bartel, B. (2005). Identification and functional characterization of Arabidopsis PEROXIN4 and the interacting protein PEROXIN22. Plant Cell 17, 3422–3435.

Zolman, B. K., Yoder, A., and Bartel, B. (2000). Genetic analysis of indole-3-butyric acid responses in Arabidopsis italiana reveals four mutant classes. Genetics 156, 1323–1337.