Review

Arabidopsis thaliana—A model organism to study plant peroxisomes

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Abstract

In higher plants, peroxisomes have been believed to play a pivotal role in three metabolic pathways, which are lipid breakdown, photorespiration and H₂O₂-detoxification. Recently, significant progress in the study of plant peroxisomes was established by forward/reverse-genetics and post-genomic approaches using Arabidopsis thaliana, the first higher plant to have its entire genome sequenced. These studies illustrated that plant peroxisomes have more diverse functions than we previously thought. Research using Arabidopsis thaliana is improving our understanding of the function of plant peroxisomes.

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Keywords: Arabidopsis; Glyoxysome; Leaf peroxisome; Lipid metabolism; Photorespiration

1. Introduction

From the mid 1980s, Arabidopsis thaliana (referred to herein as Arabidopsis) became an essential model plant for plant scientists due to the availability of various information and tools, such as whole genome sequence, molecular genetic markers and large collections of sequence-indexed DNA-insertion mutants, in addition to the ease of generating transgenic plants. This is also the case for scientists engaged in studying plant peroxisomes. In this review, we briefly summarize the unique features of plant peroxisomes, and then describe the recent progress achieved by introducing Arabidopsis as tools for forward/reverse-genetics and post-genome approaches.

2. Unique features of plant peroxisomes

Almost four decades have passed since the first discovery of plant peroxisomes. Much of our present knowledge of plant peroxisomes was established in the first era of research using biochemical and morphological techniques [1–3]. These studies clearly showed that peroxisomes in higher plants have distinct features compared to other organisms, although they also share some common features such as detoxification of H₂O₂ by catalase. One of the remarkable features of peroxisomes in higher plants is the plasticity of their functions. Plant peroxisomes are known to differentiate in function depending on the cell type. Therefore, they are subdivided into three different classes, namely glyoxysomes, leaf peroxisomes and unspecialized peroxisomes. Additionally, in some plant species ureide metabolism takes place in peroxisomes.

Glyoxysomes are present in cells of storage organs, such as endosperms and cotyledons, during post-germinative growth of oil-seed plants, as well as in cells of senescent organs [2,4]. They play an important role in lipid metabolism (Fig. 1). In dry seeds, large amounts of triacylglycerols accumulate as reserved lipids in organelles called oil bodies. Triacylglycerols in oil bodies mainly contain long-chain fatty acids such as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) [5]. During the post-germinative growth of the seedlings, fatty acids released from the triacylglycerols are metabolized to produce sucrose. Sucrose provides a carbon source that is necessary for growth before the plants begin photosynthesis. The conversion of fatty acids to succinate takes place in the glyoxysomes via fatty acid β-oxidation and the glyoxylate cycle (Fig. 1). It is worth noting that the glyoxysome is the sole (or at least predominant) site of fatty acid β-oxidation in plants, and completely degrades fatty acids into acetyl CoA by the action of acyl CoA oxidases with various substrate specificities involving short-chain specific acyl...
CoA oxidase.

Fatty acid β-oxidation and glyoxylate cycle are discussed in detail elsewhere in this volume.

Leaf peroxisomes are widely found in cells of photosynthetic organs, such as green cotyledons and leaves [9]. In C3 plants, these organs have a light-dependent O2 uptake and CO2 release called photorespiration. This physiological phenomenon is initiated by the oxygenase reaction of ribulose bisphosphate carboxylase/oxygenase (RuBisCO; a key enzyme for CO2 fixation in photosynthesis) that depends on the O2 concentration and light intensity. Two phosphoglycolates, byproducts of the oxygenase reaction, are converted to produce one phosphoglycerate, an intermediate of the Calvin–Benson cycle, and one CO2 by the photorespiratory glycolate pathway. This pathway involves many enzymatic reactions located in leaf peroxisomes, chloroplasts and mitochondria. Within the entire photorespiratory glycolate pathway, leaf peroxisomes possess glycolate oxidase, hydroxypyruvate reductase and some aminotransferases (Fig. 2). By the combination of these enzymes, leaf peroxisomes convert glycolate to glycine and serine to glycerate. Photorespiration is also discussed in detail elsewhere in this volume.

Glyoxysomes, leaf peroxisomes and unspecialized peroxisomes are known to interconvert in their functions between each other during certain cellular processes. The functional transformation of plant peroxisomes has been most extensively studied using oil seed plants. For example, reversible interconversion between glyoxysomes and leaf peroxisomes is observed during greening and senescence of the cotyledonal cells [10–14]. When the seeds germinate, seedlings start to grow using the seed reserve substances in etiolated cotyledons. After the seedlings grow and are then irradiated, the etiolated cotyledons become green and produce energy by photosynthesis. To support the drastic metabolic change, glyoxysomes are directly transformed into leaf peroxisomes during the greening process of the cotyledons. Once seedlings expand their leaves, green cotyledons gradually undergo senescence. With the process of senescence, reverse transformation from leaf peroxisomes to glyoxysomes occurs in the cotyledonal cells. Induction of glyoxysomes from leaf peroxisomes is also found in cells of other senescent organs, such as leaves and petals.

It was fortunate that defects in glyoxysomal and leaf peroxisomal function in Arabidopsis mutants could be easily visualized (Fig. 3, see in detail below). Due to this reason, Arabidopsis became the most frequently used plant for studying plant peroxisomes. Forward-/reverse-genetics and post-genome approaches using Arabidopsis are opening up a second era of research for plant peroxisomes.

3. Forward genetic analyses

One of the significant contributions of Arabidopsis is the isolation and characterization of peroxisome-defective mutants by forward genetics. In 1980, sat, the first plant mutant with a defect in peroxisomal function, was identified from a collection of mutants with defects in the photorespiratory glycolate pathway [15]. The screening procedure for the collection was based on the observation that plants still show normal or even enhanced growth in an atmosphere containing a relatively high CO2/O2 ratio that inhibits photorespiration. The expectation was that mutant plants with reduced photorespiration would show conditional defects in growth depending on the CO2 concentration.
visualized by the phenotype of high CO2 requirement (C). pex5i, and pex7i and their parental plant (WT) were grown for 8 weeks in a normal atmosphere (air) or in an atmosphere containing 1% CO2 (CO2) under constant illumination. pex5i, with defective leaf peroxisomal function, showed growth defects in air (pex5i/air), which can be complemented in the presence of high concentrations of CO2 (pex5i/CO2). Arrows indicate the top of an inflorescence apex.

conditional growth defect recognized under certain CO2 concentrations. Identification and analyses of the mutant provided the first experimental evidence that the photorespiratory glycolate pathway plays an important role, i.e., it removes the excess reducing power produced under certain photosynthetic conditions such as low CO2/O2 ratio and high light irradiation [17].

Since 1998, several Arabidopsis mutants with defects in the glyoxysomal fatty acid β-oxidation have been reported. These mutants identified by forward genetics were primarily identified by their resistance to a toxic level of 2,4-dichlorophenoxybutyric acid (2,4DB), or indole-3-butyric acid (IBA) [8,19]. The concept of the screening is based on experiments showing degradation of non-toxic 2,4DB into a toxic auxin analogue, 2,4D, by the action of fatty acid β-oxidation. Because 2,4D inhibits root elongation of Arabidopsis at an early stage of seedling growth, one can assume that 2,4D also inhibits the root elongation of wild-type Arabidopsis by its conversion to 2,4D, whereas the mutants that have defects in glyoxysomal fatty acid β-oxidation would no longer produce a toxic level of 2,4D from 2,4DB. Indeed, wild-type Arabidopsis seedlings showed growth inhibition on growth media containing an appropriate concentration of 2,4DB, whereas the mutants that have defects in glyoxysomal fatty acid β-oxidation showed 2,4DB resistance (Fig. 3A). Since glyoxysomal fatty acid β-oxidation is a predominant metabolic pathway for supplying sucrose that is necessary for germination (Fig. 1), these mutants required an exogenous supply of sucrose for germination (Fig. 3B). This screening procedure allowed the identification of mutants, such as ped1, ped2, ped3/pxa1/cts, acx1, acx3, chy1, pex4, pex5 and pex6 [8,20–28]. Proteins encoded by these defective genes can be classified as either peroxisomal proteins (Table 1) or peroxins (Table 2).

The PED3 gene [20], which is also known as PXA1 and CTS [23,28], encodes an integral glyoxisomal membrane protein, which has the typical characteristics of a “full-size” ATP-binding cassette (ABC) transporter with a domain organization of TMD (transmembrane domain)–NBD (nucleotide binding domain)—TMD–NBD. Analyses of the ped3 mutant indicate that this transporter predominantly contributes to the activity of peroxisomal fatty acid β-oxidation by transporting fatty acids across the peroxisomal membrane (Fig. 1). Fatty acids imported into peroxisomes are esterified by acyl-CoA synthetases that exist inside the glyoxysome and then catabolized by the action of fatty acid β-oxidation [29,30]. Therefore, the ped3 mutant requires concentration in the atmosphere (see Fig. 3C). This screening procedure was fruitful, and seven different alleles were identified [16].

Of these alleles, the sat mutant accumulated serine and glycine as end products of photosynthesis, mostly at the expense of starch and sucrose. Because photorespiration is the major source of serine and glycine in photosynthesizing tissue, the accumulation of these amino acids in the mutant suggested a block in the photorespiratory glycolate pathway immediately after serine, in a reaction that is catalyzed by the peroxisomal enzyme, serine-glyoxylate aminotransferase (Fig. 2, Table 1). Indeed, analysis of leaf extracts indicated that the mutants had lost the activity of this enzyme.

However, this pioneering work failed to identify/clone the SAT gene. About 20 years after the isolation of the sat mutant, a cDNA encoding leaf peroxisomal alanine-glyoxylate aminotransferase (AGT1) was isolated [18]. Analyses of the AGT1 locus indeed encodes leaf peroxisomal serine-glyoxylate aminotransferase (Fig. 2). Because of the defect in leaf peroxisomal function, sat mutants cannot metabolize phosphoglycolate by the photorespiratory glycolate pathway, and are unable to maintain sufficient activity of the Calvin–Benson cycle under a normal atmosphere. The reduced activity of the Calvin–Benson cycle may cause the conditional growth defect recognized under certain CO2 concentrations. Identification and analyses of the mutant provided the first experimental evidence that the photorespiratory glycolate pathway plays an important role, i.e., it removes the excess reducing power produced under certain photosynthetic conditions such as low CO2/O2 ratio and high light irradiation [17].

Since 1998, several Arabidopsis mutants with defects in the glyoxysomal fatty acid β-oxidation have been reported. These mutants identified by forward genetics were primarily identified...
half-size ABC transporters, which mediate the transport of long-chain fatty acids from peroxisomes to the cytosol. The structural difference between PED3 products with yeast and mammal counterparts may be due to the difference of substrate specificity, since it seems that PED3 products transport not only long-chain fatty acids derived from seed reserved lipids but also precursors of some plant hormones such as IBA and OPDA (see in detail below) [31].

One of the advantages of forward genetics has been the ability to determine which loci predominantly contribute to glyoxysomal fatty acid β-oxidation. A good example of this is the ped1 mutant [8,32]. Molecular genetic analyses revealed that the PED1 gene encodes 3-ketoacyl-CoA thiolase, an enzyme in the final step of the fatty acid β-oxidation (Fig. 1, Table 1). Immunoblot analysis indicated that the ped1 mutant lacks a detectable amount of 3-ketoacyl-CoA thiolase in glyoxysomes. Bioinformatic analysis suggested that there exist other two isogenes for the enzyme (KAT1 (At1g04710), KAT5 (At5g48880)) in the genome [32]. However, it is evident that the PED1 gene product predominantly contributes to the production of sucrose from seed reserved lipids during germination because the mutant showed severe growth inhibition in the absence of exogenously supplied sucrose. Because of the defect, the ped1 mutant had enlarged glyoxysomes containing tubular structures formed by invaginations of glyoxysomal membrane [33]. The tubular structures contained small vesicles that may have been derived from oil bodies. These morphological observations may reflect an unknown mechanism for incorporating the fatty acids from oil bodies to glyoxysomes as substrates for fatty acid β-oxidation.

Acyl-CoA oxidase is another enzyme involved in glyoxysomal fatty acid β-oxidation. Six ACX genes (ACX1–6) were predicted by bioinformatic analysis, and substrate specificities of ACX1 (long/medium-chain fatty acyl-CoA specific), ACX2 (long-chain fatty acyl-CoA specific), ACX3 (medium-chain fatty acyl-CoA specific) and ACX4 (short-chain fatty acyl-CoA specific) have been biochemically determined [6,7,25,34–37]. Of these, the acc1 and acc3 mutants were identified by their resistance to IBA. However, they could germinate without supplying exogenous sucrose. This phenotype is clearly different from that of the mutants mentioned above, and suggests that a defect in one of these isogenes could be partially complemented by the other ACX genes.

The chyl mutant was also identified from the collection of IBA-resistant mutants. CHY1 encodes a peroxisomal protein that showed sequence similarity with a mammalian β-hydroxyisobutryl-CoA hydrolase [22], an enzyme involved in

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Arabidopsis mutants with defect in peroxisome-related protein</th>
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<tbody>
<tr>
<td>Name of protein</td>
<td>Name of mutant</td>
</tr>
<tr>
<td>Photosynthetic glycolysis metabolism</td>
<td></td>
</tr>
<tr>
<td>Serine-glyoxylate</td>
<td>sat</td>
</tr>
<tr>
<td>Aminotransferase</td>
<td></td>
</tr>
<tr>
<td>Glutamate-glyoxylate</td>
<td>aoat1</td>
</tr>
<tr>
<td>Aminotransferase</td>
<td>aoat2</td>
</tr>
<tr>
<td>Fatty acid degradation</td>
<td></td>
</tr>
<tr>
<td>Full-size ABC transporter</td>
<td>ped3/pxa1/cts</td>
</tr>
<tr>
<td>Acyl CoA synthetase (long-chain)</td>
<td>lacs6</td>
</tr>
<tr>
<td>(similar to ACX1)</td>
<td>lacs7</td>
</tr>
<tr>
<td>Acyl-CoA oxidase (long-chain)</td>
<td>acc1</td>
</tr>
<tr>
<td>(similar to ACX3)</td>
<td>acc6</td>
</tr>
<tr>
<td>(short-chain)</td>
<td>acc4</td>
</tr>
<tr>
<td>Multifunctional enzyme</td>
<td>aim1</td>
</tr>
<tr>
<td>3-ketoacyl CoA thiolase</td>
<td>ped1/kat2</td>
</tr>
<tr>
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<td>mls</td>
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<tr>
<td>Citrate synthase</td>
<td>csy2</td>
</tr>
<tr>
<td>(similar to ACX3)</td>
<td>csy3</td>
</tr>
<tr>
<td>Isoctiritate lyase</td>
<td>icl</td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
<tr>
<td>Dynamin 3A</td>
<td>apm1</td>
</tr>
<tr>
<td>3-hydroxyisobutyry-CoA hydrolase</td>
<td>chyl</td>
</tr>
<tr>
<td>12-oxophytodienoid acid reductase</td>
<td>dde1/opr3</td>
</tr>
</tbody>
</table>

(T-DNA)

FG, forward-genetical screening from a collection of EMS-mutagenized mutants; FG(T-DNA), forward-genetical screening from a collection of T-DNA inserted mutants; RG, reverse-genetical identification of a DNA-inserted knockout mutant.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>List of Arabidopsis PEX gene orthologues and mutants</th>
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<tbody>
<tr>
<td>Name</td>
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</tr>
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<td>PEX1</td>
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<tr>
<td>PEX2</td>
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<tr>
<td>PEX3.1</td>
<td>At1g48640</td>
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<tr>
<td>PEX3.2</td>
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<tr>
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<td>At2g45690</td>
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<td>PEX17</td>
<td>At4g18197</td>
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<tr>
<td>PEX19.1</td>
<td>At3g03490</td>
</tr>
<tr>
<td>PEX19.2</td>
<td>At5g17550</td>
</tr>
<tr>
<td>PEX22</td>
<td>At3g21865</td>
</tr>
</tbody>
</table>

FG, forward-genetical screening from a collection of EMS-mutagenized mutants; FG(T-DNA), forward-genetical screening from a collection of T-DNA inserted mutants; RG, reverse-genetical identification of a DNA-inserted knockout mutant; RG(RNAi), knockdown mutant produced by RNA interference.
catabolism of branched amino acids including valine, leucine and isoleucine. Interestingly, the mammalian orthologue of this enzyme is localized in mitochondria. The subcellular localization of the branched chain amino acid’s catabolism in plants remains controversial, but the existence of the enzyme in peroxisomes strongly supports the idea that branched amino acid catabolism occurs (at least partially) in peroxisomes in higher plants.

As it is clearly and elegantly described in the other sections of this issue, our knowledge of peroxisome biogenesis has been much improved by identification/characterization of peroxins. Arabidopsis mutants identified by the forward genetic screening also made a concrete contribution to this field. The genes identified from these mutants were PEX4, PEX5, PEX6, PEX12, PEX13 and PEX14 (Table 2). Among them, the plant PEX14 gene has been identified from analyses of the Arabidopsis ped2 mutant, which was originally isolated by its resistance to a toxic level of 2,4-D [21]. The requirement of high CO2 concentration in the adult phase and that of exogenously supplied sucrose during germination indicated that the mutant has defects in not only glyoxysomes but also leaf peroxisomes. Indeed, examination of cells in all organs, i.e. etiolated cotyledon, green cotyledon, leaf and root, shows peroxisomes with abnormal morphology. The pleiotropic defects of the ped2 mutant suggested that the ped2 gene product regulates peroxisomal functions through a mechanism common for all types of plant peroxisomes. Subsequent analyses revealed that the defective gene encodes AtPex14p, a 75-kDa peroxisomal membrane protein, which is similar to human Pex14p, a product of the PEX14 gene [38,39]. AtPex14p is involved in the import of nascent polypeptides containing either one of two peroxisomal targeting signals known as PTS1 and PTS2 (Fig. 4, see detail below). Loss of AtPex14p inhibits the import of both PTS1-containing and PTS2-containing proteins into peroxisomes, and reduces peroxisomal functions in all cell types.

As is the case in yeasts and mammals, however, neither PTS1 nor PTS2 directly binds with AtPex14p. Instead, it has been shown that PEX5 and PEX7 gene products, AtPex5p and AtPex7p, function as specific receptors recognizing PTS1 and PTS2, respectively [40]. The tetrapeptide repeat (TPR) in AtPex5p binds with PTS1, while the WD40 repeat in AtPex7p binds with PTS2. The Arabidopsis PEX5 gene produces a single transcript. It differs from the mammalian PEX5 gene, which produces two functionally different products by alternative splicing [41]. AtPex5p and AtPex7p form a PTS1/PTS2 receptor complex by binding between the amino-terminal domain of AtPex5p and the carboxy-terminal domain of AtPex7p. Nascent polypeptides containing either PTS1 or PTS2 bind with tetrapeptide repeat (TPR) in AtPex5p and the WD40 repeat in AtPex7p, respectively. After the formation of the receptor–cargo complex, the two amino-terminal domains (S1–S6L and R6–R9R) of AtPex14p capture the receptor–cargo complex by binding it to WXXXY/Y repeat that exists in the middle of AtPex5p [40]. These results indicate that AtPex5p and AtPex14p mediate import of both PTS1- and PTS2-containing proteins, while AtPex7p carries out the role of transferring PTS2-containing proteins to the import pathway (Fig. 4). The biochemical characteristics of the AtPex5p explain the unique phenotype of the Arabidopsis pex5-1 mutant [19,26]. Despite the defect in the PEX5 gene, the pex5-1 mutant was defective in the import of PTS2-containing proteins, but not in the import of PTS1-proteins. The pex5-1 mutant caused amino acid substitution of S189L in AtPex5p. This lesion existed in a domain necessary for its binding with AtPex7p but not import of PTS1-containing proteins. It suggests that the defective AtPex5p could function as a PTS1 receptor without binding with AtPex7p, but lost its ability to import PTS2-containing proteins through its binding with AtPex7p. Mutants defective in PEX4 and PEX6 have also been identified [24,27]. The defect in PEX6 caused reduced numbers of enlarged peroxisomes, while analysis of the pex4-1 mutant allowed identification of its binding protein, AtPex22p, a protein with conserved size and predicted topology compared to yeast Pex22p but with very low amino acid sequence identity.

Arabidopsis apm mutants showing aberrant morphology of peroxisomes have been identified from point-mutated M2 seedlings of whose transgenic parental lines express a chimeric protein, GFP-PTS1 [42]. In cells of the parental plant, GFP-PTS1 was correctly recognized by the peroxisomal protein import machinery, allowing visual confirmation of peroxisomes with normal size and number by its fluorescence without killing the plant [43]. Two mutants, apm2 and apm4, have been identified by partial mislocalization of GFP-PTS1 in the cytosol. Molecular genetic analyses of these mutants revealed that APM2 and APM4 encode AtPex13p and AtPex12p, respectively [44]. In contrast, the apm1 mutant has been identified by abnormal
morphology of peroxisomes [45]. Peroxisomes in the mutant were longer and reduced in number compared to wild type. This may be caused by inhibition of peroxisomal division. Indeed, APM1 encodes DRP3A (dynamin-related protein 3A). DRP3A was associated with not only peroxisomes but also mitochondria. In agreement with this result, morphologies of not only peroxisomes, but also of mitochondria become aberrant in the apm1 mutant.

There exists a certain number of peroxisome-related Arabidopsis mutants that have been identified by forward genetic screening which was not focused on peroxisomes. These unexpected results are bringing us a new insight into unknown plant peroxisomal functions. One of the examples is the aim1 mutant (Table 1). This mutant was originally identified by its wide range of morphological defects associated with reproductive development, i.e. abnormal inflorescence and floral development. Genetic analyses revealed that the AIM1 gene encodes a protein showing extensive similarity to the cucumber multifunctional protein, one of the enzymes for fatty acid \( \beta \)-oxidation [46]. This conclusion is supported by the results showing that the aim1 mutant is resistant to a toxic level of 2,4D, and that its leaf cells have altered fatty acid composition. Indeed, a recombinant protein produced from the AIM1 cDNA has corresponding enzyme activity. These data suggest that peroxisomal fatty acid \( \beta \)-oxidation is also involved in determining reproductive development. It is worth mentioned that MFP2, another gene encoding the multifunctional protein, was recently identified [47]. Function of MFP2 products obviously plays a dominant role during germination, since the mfp2 mutant requires exogenous supply of sucrose for germination without showing morphological defects in reproductive development. Another line of evidence that suggests the involvement of peroxisomal fatty acid \( \beta \)-oxidation in morphogenesis came from the analysis of ped1 and ped3 mutants [20]. As described above, a single mutation in either PED1 or PED3 genes does not show any obvious morphological defect in adult plants. However, once these two mutations were introduced into the same plant, the ped1/ped3 double mutant had wavy leaves with irregular shapes. The double mutant had difficulty to develop inflorescences, and those that did develop were dwarfed and had abnormal structure. Although the inflorescences had some flowers, they were sterile.

The role of peroxisomal fatty acid \( \beta \)-oxidation in determining the morphology of the adult plants is unknown. One possible explanation is the production of signaling molecule(s) by plant peroxisomes. In this context, it is worth mentioning analyses of two independently identified male-sterile Arabidopsis mutants, dde1 and opr3 [48,49]. The male-sterile phenotype can be rescued by exogenous application of jasmonic acid. The DDE1/OPR3 gene encodes a third enzyme of 12-oxoxytydienoic acid reductase (OPR3). Two other cytosolic proteins, OPR1 and OPR2, with OPR activities have been identified previously. However, comparison of enzymatic properties between OPR3, OPR1 and OPR2, indicated that OPR3 is an isozyme related to jasmonate biosynthesis [45,46]. OPR3 contains a peroxisomal targeting signal (PTS1) at the carboxyl terminus. There is no such signal in either OPR1 or OPR2. Indeed, peroxisomal localization of OPR3 has been demonstrated by immunocytochemical analysis and GFP-OPR3 fusion proteins [50]. These data indicate that peroxisomes are the site of reactions catalyzed by OPR3, and that subsequent fatty acid \( \beta \)-oxidation is required for the biosynthesis of jasmonic acid.

The plant PEX16 gene was identified using a mutant called ssel [51,52]. The ssel seeds shrank upon desiccation occurring during late embryogenesis. The homozygous ssel plant produces 90% shrunken seeds and 10% normal round seeds. The shrunken seeds are not viable. The SSE1 cDNA encodes APEX16p, a protein similar to a product of the PEX16 gene identified in yeast, Yarrowia lipolytica [53]. The SSE1 product localizes in plant peroxisomes, and partially complements the phenotype of yeast pex16. Arabidopsis is a typical oil-seed plant. Therefore, cells of the mature seeds contain protein bodies and oil bodies, the sites of accumulation for storage proteins and reserved lipids, respectively. The ssel seeds, however, contain a few oil bodies and no recognizable protein bodies. By contrast, they accumulate starch granules in amyloplasts, membrane stacks, vesicles and vacuoles. The reason why the defect in the SSE1 (APEX16) gene results in accumulation of excess starch over lipids and proteins in seeds is beyond our present knowledge of plant peroxisomal functions. In any case, this mutant suggests that plant peroxisomes still possess unidentified function(s), which affect the formation of protein bodies, oil bodies and amyloplasts.

Analysis of a photomorphogenesis mutant (det1) also eventually revealed a connection with peroxisomes. The Arabidopsis DET1 protein has been demonstrated to be a repressor of photomorphogenesis, since dark-grown det1 mutants develop like light-grown plants, i.e., short hypocotyls, opened cotyledons with developed chloroplasts [54]. Hu et al. [55] recently identified ted3 as a dominant suppressor of det1 phenotypes. Developmental defects and the abnormal expression of many genes in det1 are rescued by ted3. ted3 also partially suppresses phenotypes of another pleiotropic deetiolated mutant cop1. TED3 encodes a 38-kDa peroxisomal protein that is most similar to yeast and mammalian Pex2p, a PEX2 gene product. Pex2p is an integral peroxisomal membrane protein containing a cytoplasmically exposed zinc RING finger domain, and is thought to be involved in the translocation process of proteins across membrane [56]. Hu et al. [55] analyzed the peroxisomal function of det1 mutants and det1/ted3 double mutants, extensively, and concluded that det1 seedlings have defective peroxisomes, a trait that can be rescued by the ted3 gain-of-function mutation. These findings suggest that plant peroxisomes are involved in the regulation of photomorphogenesis, which is negatively controlled by DET1 and COPI genes.

4. Reverse genetic analyses

Along with the Arabidopsis whole genome sequencing project [57], much information such as predicted genes, open reading frames, proteins and their functions have become available from public domains. In parallel, tremendous efforts have been made to prepare large scale collections of transposon (Ds)- and T-DNA (transfer DNA of Agrobacterium tumefaci-
ciens)-insertion mutants, and databases listing the defective gene for each mutant (for example [58]). Accumulation of this information makes reverse genetic approaches more feasible than ever. Several attempts have been made to study plant peroxisomes using reverse genetics (Tables 1 and 2).

One good example of this is a reverse genetic study of glyoxyosomal long-chain acyl-CoA synthetase [29,30]. The enzyme had been biologically characterized using biochemical and molecular techniques. There are two isogenes, LACS6 and LACS7, encoding the enzyme in the Arabidopsis genome. By a reverse genetic approach, T-DNA inserted knockout mutants, namely lacs6-1 and lacs7-1, were isolated and characterized [59]. No obvious phenotype was found in mutants defective in each single gene. However, the lacs6-1/lacs7-1 double mutant abolished long-chain acyl-CoA synthetase activity, and required exogenous supply of sucrose for germination. This result suggested that LACS6 and LACS7 have a redundant function. It clearly indicated that the reverse genetic approach has much potential in the study of plant peroxisomes. Similar approaches has been made to characterize peroxisomal proteins that cannot be identified by corresponding mutants, such as glutamate-glyoxylate aminotransferase (AOAT1, AOAT2) [60], acyl-CoA oxidase (ACX2, ACX4, ACX5 and ACX6) [25] and glyoxyosomal enzymes (MLS, CSY2, CSY3 and ICL) [61–63].

Because of the importance of peroxins in understanding biogenesis of plant peroxisomes, Arabidopsis PEX genes have been predicted bioinformatically. At present, 22 PEX genes encoding 16 types of peroxins were identified/predicted (Table 2) [64,65]. The reverse genetic approach was shown to be applicable for characterization of the predicted PEX genes. Of these, the pex10 mutant has been identified solely by reverse genetics (Table 2) [66,67]. Interestingly, the homozygous knockout mutant showed an embryonic lethal phenotype. The embryonic lethal phenotype is not limited to the pex10 mutation. Indeed, other loss-of-function pex mutants, i.e. pex2, pex12 and pex16 also showed severe defects in embryos, suggesting the importance of plant PEX gene function for embryo viability [51,55,68]. It is, however, difficult to extensively apply the DNA-inserted knockout mutants to the study of plant peroxisomes, because the embryonic lethality prohibits analyses of peroxisomes in cells of adult plants showing the phenotype.

One possible solution is analyses of knockdown mutants generated by RNA interference. Double-stranded RNA interference has been shown to be an effective trigger of gene silencing in a number of organisms including Arabidopsis [69]. Arabidopsis knockdown mutants can be generated by transformation using a DNA construct specifically designed to induce sequence-specific RNA degradation by RNA interference. It had been reported that this method could efficiently induce specific and heritable gene silencing in a number of Arabidopsis genes [70–72]. To analyze the function of PEX5 and PEX7 in vivo, knockdown mutants of these genes, i.e. pex5i and pex7i, have been generated by RNA interference (Fig. 3, Table 2) [73]. In spite of the fact that pex5i and pex7i showed severe phenotypes and that the amount of both AtpPex5p and AtpPex7p in the cells was significantly reduced, these knockdown mutants were not embryonic lethal due to the “partial” loss-of-function phenotype. Analyses of the pex5i and pex7i mutants revealed following two points: (1) Glyoxysomal function is regulated by both AtpPex5p and AtpPex7p, while leaf-peroxisomal function is regulated by AtpPex5p, but not AtpPex7p (Fig. 3). (2) AtpPex5p is involved in import of both PTS1-containing and PTS2-containing proteins, whereas AtpPex7p is involved in import of only PTS2-containing proteins. These results suggest that PEX5 and PEX7 differentially contribute the maintenance/differentiation of peroxisomal functions in plants. In addition, recent publications on PEX11 and PEX19 suggest alternative approach with overexpressed plant PEX gene [74,75].

5. Post-genome approaches

Since the whole Arabidopsis genome sequence has been identified, one can search for all the genes that encode peroxisomal proteins having either PTS1 ([C/A/S/P]-[K/R/H]-[L/M] at the carboxy-terminal end) or PTS2 (R-[A/I/Q/L]-x(5)-H-[L/F] at the amino-terminal domain) [76,77]. The result of this search indicates that 182 genes may encode PTS1-containing proteins, while 74 genes may encode PTS2-containing proteins. Addition of known peroxisomal proteins without the targeting signals (i.e., catalase and peroxisomal membrane proteins) gave a total number of 286 peroxisomal genes. From the sequence similarity of the products, they can be classified into 224 gene families [78]. The functions of only some of these gene families have been experimentally determined. Another search algorithm allowed identification of more candidates for plant peroxisomal proteins [79,80]. This survey of Arabidopsis genes allows for transcript analysis of peroxisomal gene expression. Organ-specific expression of peroxisomal genes was comprehensively examined by making DNA microarrays covering all the candidates for Arabidopsis peroxisomal genes [78]. Clustering analysis of data obtained from the transcriptomic analysis indicated peroxisomal genes could be divided into five groups. Genes involved in one group showed ubiquitous expression in all organs examined. In contrast, genes involved in the other four groups were classified as showing organ-specific expression in seedlings, cotyledons, roots and both cotyledons and leaves. These data propose an idea that plant peroxisomes are diversely differentiated into not only glyoxy- somes and leaf-peroxisomes, but also cotyledonal peroxisomes, root peroxisomes.

Completion of Arabidopsis whole genome sequencing also allowed proteomic analysis of peroxisomal proteins. A number of proteins have been determined after separation of proteins in highly purified glyoxysomes and leaf-peroxisomes by 2D gel electrophoresis, and subsequent peptide MS finger printing [81,82]. Some of these were identified as unknown proteins, and were successively investigated. Of these, GPK1 contained a conserved protein kinase domain with a possible peroxisome targeting signal at its carboxyl termini, and was localized on the glyoxysomal membrane. These results suggested that protein phosphorylation/dephosphorylation inside the peroxisomes may be involved in regulating peroxisomal functions in plants. Overall, conclusions obtained from post-genomic-sequencing approaches coincide with the evidence obtained from the
peroxisome-related mutants, which indicate the existence of a greater diversity of functions in plant peroxisomes than we previously thought.

6. Perspectives

As described above, the application of Arabidopsis as a genetic tool significantly contributed to our knowledge of the functional divergence and biogenesis of plant peroxisomes. However, interesting features of plant peroxisomes that have been established by biochemical/cell biological techniques during the first era of research, such as the regulatory mechanism of functional transition, still remain obscure. It is now time to combine the technical advantages of the first and second eras. Combining the genetic and post-genomic-sequencing analyses with biochemical/cell biological analyses will open the door to a third era of research on plant peroxisomes.

References


