Transcriptome Analysis of Peroxisomal Differentiation and the Functional Transition in *Arabidopsis thaliana*

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Abstract

Peroxisomes in higher plant cells have been recognized to differentiate into at least three different classes, namely glyoxysomes, leaf peroxisomes and unspecialized peroxisomes. Glyoxysomes are present in cells of storage organs, such as cotyledons during post-germinative growth of oil seed plants, as well as in senescent organs. They contain enzymes for β-oxidation and the glyoxylate cycle to degrade fatty acids. In contrast, leaf peroxisomes are found widely in cells of photosynthetic organs. They play a role of glycolate metabolism in photorespiration pathway. Other organs, such as roots and stems, contain unspecialized peroxisomes whose function is still obscure. Those differentiated peroxisomes are known to convert into one another under certain conditions. For example, glyoxysomes in etiolated cotyledons are transformed directly into leaf peroxisomes during the greening of cotyledons. Recent progresses on the analyses of Arabidopsis mutants suggest that plant peroxisomes have more diverse functions than we know at present.

In chapter 1, to reveal genetic configuration of plant peroxisomes, I comprehensively surveyed genes related peroxisomal function and biogenesis in the entire Arabidopsis genome sequence. In this survey, I used amino acid sequences that are well known as targeting signals of peroxisomal matrix proteins, namely PTS1 and PTS2. I identified 256 gene candidates of PTS1- and PTS2-containing proteins. In addition to the 256 gene candidates, I put in another 30 genes of non-PTS-containing proteins predicted to relate peroxisomal function and biogenesis, such as peroxisomal membrane proteins. Of these, only 29 proteins have been reported to be functionally characterized as peroxisomal proteins in higher plants. Using the total 286 peroxisomal genes, I extensively investigated expression profiles in various organs of Arabidopsis to reveal diversity of plant peroxisomes. Statistical analyses of these expression profiles revealed that peroxisomal genes could be divided into five groups. One group showed ubiquitous expression in all organs examined, while the other four were classified as showing organ-specific expression in seedlings, cotyledons, roots and in both cotyledons and leaves. These data proposed more detailed description of differentiation of plant peroxisomes.

The aims of chapter 2 are to reveal gene expression profiles during functional transition of plant peroxisomes and to investigate candidate genes functioned at the process. I compared gene expression profiles of cotyledons illuminated for 2hr, 6hr, 12hr and 24hr in white light following 4 days growth in dark with cotyledons grown for 4 days in dark. During illumination, the genes of enzymes for β-oxidation and glyoxylate cycle were reduced. On the other hand, genes of enzymes for phtorespiration were induced by light. Along with these genes, a lot of peroxisomal genes changed their expression levels during irradiation. In contrast, nine peroxisomal genes showed transitory expressed pattern during illumination. It was suggested that the genes functioned at the process of functional transition of peroxisomes.

In this study, I revealed the diversity of plant peroxisomes and declared gene expression profiles during the functional transition of peroxisomes. This study will serve as a foundation for revealing unidentified peroxisomal functions in plant cells.

Contents

Abbreviations

ACX1	: medium to long chain acyl-CoA oxidase
ACX2	: long-chain acyl-CoA oxidase
ACX3	: medium-chain acyl-CoA oxidase
ACX4	: short-chain acyl-CoA oxidase
AGT1	: alanine:glyoxylate aminotransferase 1
BCAA	: blanched chain amino acid
cDNA	: complementary DNA
CHY1	: β-hydroxyisobutyryl-CoA hydrolase 1
CoA	: coenzyme A
CS	: citrate synthase
Cy3	: cyanine 3
Cy5	: cyanine 5
EST	: expressed sequence tag
GGT1	: glutamine:glyoxylate aminotransferase 1
GGT2	: glutamine:glyoxylate aminotransferase 2
GO	: glycolate oxidase
HEPES	: N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
H_2O_2	: hydrogen peroxide
HPR	: hydroxypyruvate reductase
IAA	: indole-3-acetic acid
IBA	: indole-3-butyric acid
ICL	: isocitrate lyase
IgG	: immunoglobulin G
KAT	: 3-ketoacyl-CoA thiolase
LACS	: long-chain acyl-CoA synthetase
Log	: logarithm
MDH	: malate dehydrogenase
MFP	: multifunctional protein
mPTS	: membrane peroxisomal targeting signal
MS medium	: Murashige and Skoog medium
NADP	: reduced nicotinamide adenine dinucleotide phosphate

NADPH	: nicotinamide adenine dinucleotide phosphate
NOS	: nitric oxide synthase
ORF	: open reading frame
PCR	: polymerase chain reaction
PED	: peroxisome diffective
PEX	: peroxin
PMP	: peroxisomal membrane protein
PTS	: peroxisomal targeting signal
ROS	: reactive oxygen species
RT-PCR	: reverse transcription of RNA followed by PCR
RubisCO	: ribulose-1,5-bisphosphate carboxylase/oxygenase
Ru1.5BP	: ribrose-1,5-bisphosphate
SD	: standard deviation
SDS	: sodium dodecyl sulfate
SOD	: superoxide dismutase
TCA cycle	: tricarboxylic acid cycle

General Introduction

1. Peroxisomes in higher plants

Peroxisomes are globular organelles, approximately 1 μm in diameter, and ubiquitously found in eukaryotic cells. It has been characterized that they have a high density (1.25 g/cm^3, compared with 1.18 g/cm^3 for mitochondria) and contain catalases as a marker enzyme. Peroxisomes in higher plant cells are known to differentiate in function depending on the cell type. Because of the functional differentiation, plant peroxisomes have been subdivided into at least three different classes, glyoxysomes, leaf peroxisomes and unspecialized peroxisomes (Beevers, 1979). Glyoxysomes are present in cells of storage organs, such as endosperms and cotyledons during the post-germinative growth of oil-seed plants, as well as in senescent organs (Bellis and Nishimura, 1991). They play an important role for fatty acid degradation. Leaf peroxisomes are widely found in cells of photosynthetic organs and play a part in photorespiratory pathway (Tolbert et al., 1968; Nishimura et al., 1996). These organelles contain distinct sets of enzymes that lead to different metabolic roles. Other organs, such as roots and stems, contain unspecialized peroxisomes whose function is still obscure.

2. Function of plant peroxisomes

Glyoxysomes in germinating oilseeds

Glyoxysomes contain enzymes responsible for fatty acid β-oxidation and the glyoxylate cycle. They play a role in fatty acid degradation. In glyoxysomes, fatty acids are firstly activated to fatty acyl-CoA by a fatty acyl-CoA synthetase (Fulda et al., 2002; Hayashi et al., 2002a). ATP is used as an energy source and to form an activating intermediate. Subsequently, fatty acyl-CoA is metabolized by fatty acid β-oxidation. Acyl-CoA oxidases (Hayashi et al., 1998a; Hayashi et al., 1999; Hooks et al., 1999; Eastmond et al., 2000a; Froman et al., 2000) catalyze the first oxidative reaction of the pathway with the conversion of fatty acyl-CoA into *trans*-2-enoyl-CoA. The *trans*-2-enoyl-CoA is converted into 3-ketoacyl-CoA by multifunctional proteins that exhibit 2-*trans*-enoyl-CoA hydratase and L-3-hydroxyacyl-CoA dehydrogenase activities (Richmond and Bleecker, 1999; Eastmond and Graham, 2000). Finally, 3-ketoacyl-CoA

thiolase (Preisig-Muller et al., 1994; Kato et al., 1996; Eastmond et al., 2000a) is responsible for the cleavage of the acetyl group from the acyl-CoA thereby creating an acyl-CoA two carbons shorter that can then re-enter the β-oxidation spiral. Acetyl-CoA, an end product of fatty acid β-oxidation, is metabolized further to produce succinate by the action of the glyoxylate cycle. The glyoxylate cycle is composed of four glyoxysomal enzymes, namely citrate synthase, isocitrate lyase, malate synthase and malate dehydrogenase, and a cytosolic aconitase (Graham et al., 1989; Gietl, 1990; Hayashi et al., 1995; Kato et al., 1995). In essence, the glyoxylate cycle is a modified form of the tricarboxylic acid (TCA) cycle that bypasses the decarboxylative steps, allowing the net production of carbon skeletons, with no carbon lost as CO_2. The isocitrate lyase and malate synthase are unique enzyme of glyoxylate cycle to bypass the decarboxylation steps of the TCA cycle. Two moles of acetyl-CoA are introduced with each turn of the cycle, resulting in the synthesis of one mole of the four-carbon compound succinate.

Leaf peroxisomes

Leaf peroxisomes are known to contain a different set of enzymes from glyoxysomes, which mediate a photorespiratory glycolate metabolism. Photorespiration is a light-dependent process that results in the uptake of O_2 and the release of CO_2. Photorespiration is linked to photosynthesis by the dual function of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) which uses the mutually competitive substrates CO_2 and O_2 or reaction ribrose-1,5-bisphosphate (Ru1.5BP). Carboxylation of Ru1.5BP produces two molecules of 3-P-glycerate, whereas oxygenation of Ru1.5BP produces one molecule of 3-P-glycerate and one of 2-P-glycolate. The phosphate group of 2-P-glycolate is hydrolyzed to glycolate by the stromal enzyme phosphoglycolate phosphatase. Glycolate is exported from the chloroplast in exchange for glycerate. Glycolate diffuses to leaf peroxisomes and probably passes through the membrane via the electrophysiologically characterized porin-like channel (Reumann et al., 1995, 1998). Glycolate is oxidized internally by the matrix enzyme glycolate oxidase (Volokita and Somerville, 1987; Tsugeki et al., 1993). It is subsequently transaminated to glycine. This transamination is achieved by at least two alternative ways, as catalyzed by serine:glyoxylate aminotransferase and glutamate:glyoxylate aminotransferase (Liepman and Olsen, 2003). The glycine is oxidized to serine in mitochondria, and

enters the peroxisomal matrix again. It is deaminated to hydroxypyruvate by a serine:glyoxylate aminotransferase (Liepman and Olsen, 2001). Hydroxypyruvate is reduced to D-glycerate by the NADH-dependent hydroxypyruvate reductase (Hayashi et al., 1996b). the membrane of leaf peroxisomes appears to be impermeable to NADH (Reumann et al.,1994). Reducing equivalents need to be imported from the cytosolic pool via malate and provide internally by the activity of peroxisomal malate dehydrogenase. The D-glycelate, leaves the peroxisome and is transported to the chloroplast stroma. Then it is catalyzed by glycerate kinase. The resulting P-glycerate can re-enter the reductive photosynthetic carbon cycle.

Reactive oxygen metabolism

Peroxisomes have an essentially oxidative type of metabolism. Peroxisomes contain enzymes producing H_2O_2, such as acyl-CoA oxidases and glycolate oxydase. The catalase is responsible for the removal of toxic H_2O_2. Catalase is exclusively localized in peroxisomes, which comprises as much as 10-25% of the total peroxisomal protein. Recently, the production of superoxide radicals ($O_2^{\cdot -}$), another reactive oxygen species (ROS), has been demonstrated in plant peroxisomes. It has also been identified antioxidative enzymes in plant peroxisomes, which including superoxide dismutases (SODs), the components of the ascorbate-glutahione cycle, several NADP-dependent dehydrogenases, and the nitric oxide-generating enzyme nitric oxide synthase (NOS) (Corpas et al., 2001). This has suggested the existence of new cellular functions for peroxisomes related to ROS metabolism, which appear to be particularly relevant in the process of leaf senescence and in different abiotic stress conditions.

3. Functional transition of plant peroxisomes

A characteristic property of plant peroxisomes is their metabolic plasticity. Peroxisomal enzymatic content can vary depending on the organism, cell/tissue-type and environmental conditions. An illustrative example of the inducible nature of peroxisomal metabolism is the light-induced transition of glyoxysomes to leaf peroxisomes (Mullen and Trelease, 1996). In germinating fatty seedlings, glyoxysomal enzymes first appear in etiolated cotyledons. When the seedlings are irradiated, glyoxysomal enzymes are degraded and leaf peroxisomal enzymes appear in cotyledons.

Likewise, during the senescence of leaves the reverse process is observed, that is the conversion of leaf peroxisomes into glyoxysomes (Nishimura et al., 1996). In the process of functional transition, peroxisomes are transformed directly into one another (Titus and Becker, 1985; Nishimura et al., 1986).

4. Peroxisomal proteins

Protein transport of matrix proteins

Peroxisomal matrix proteins are synthesized in the cytosol and function after their post-translational transport into peroxisomes (Lazarow and Fujiki, 1985). Most of the known plant peroxisomal matrix proteins have been shown to contain one of two peroxisome-targeting signals within their amino acid sequences. One type of targeting signal, PTS1, is a unique tripeptide sequence found on the carboxyl-terminal end of proteins (Hayashi et al., 1996a). Many of the known peroxisomal enzymes possess PTS1. Permissible combinations of amino acid sequences for plant PTS1 seems to be species dependent (Mullen et al., 1997; Kragler et al. 1998; Hayashi et al. 1997). In Arabidopsis, PTS1 is reported to be (C/ A/ S/ P)-(K/ R)-(I/ L/ M) (Hayashi et al. 1997). Another type of targeting signal, PTS2, is involved in a cleavable amino-terminal presequence (Gietl et al., 1994; Kato et al. 2000). The amino terminal presequences contain a consensus sequence, (R)-(A/ L /Q/ I)-X5-(H)-(I/ L/ F) (X stands for any amino acid) (Kato et al. 2000). The PTS2-containing proteins are synthesized as precursor proteins, with larger molecular mass due to the amino-terminal presequence. The amino-terminal presequence is processed to form the mature protein after its transport into the peroxisomes.

Protein transport of membrane proteins

Although it is generally accepted that peroxisomal membrane proteins (PMPs) are synthesized in the cytosol (Fujiki et al., 1984; Suzuki et al., 1987; Diestelkotter and Just, 1993; Imanaka et al., 1996), PMPs do not possess a PTS1 or PTS2. PMPs are therefore thought to be imported into peroxisomes by distinct targeting mechanisms from that used by peroxisomal matrix proteins. PMPs have been suggested to have a membrane peroxisomal targeting signal (mPTS) such as a 5 basic amino acid

like LKKKR (Dyer al al. 1996). Depending upon the protein and/or organism, the mPTS can vary from a short stretch of three to six positively charged amino acids residues to large nonoverlapping segments that do not contain obvious consensus motifs (Trelease, 2002). In contrast to the matrix proteins, mPTS have not been well characterized.

Peroxins

PEX genes can be classed as either cytoplasmic or membrane associated (integral or peripheral). During the growth and maturation of new peroxisomes, the recruitment of lipids and membrane proteins is required in order to build up the selectively permeable membrane. Although the properties of most of these PEX gene proteins (peroxins) are still being investigated, together, they perform the functions of early peroxisome biogenesis, PMP import, targeting receptors, matrix protein import, receptor recycling and proliferation. Twenty-six genes functioned for biogenesis of peroxisomes have been isolated from various organisms. Many of them are present in mammals, plants, and invertebrates, although the peroxisomal functions differ among these organisms. These genes have been given the acronym "PEX" and the corresponding encoded protein "peroxin" (Distel et al., 1996). Genetic screens have identified mutants in five of them in Arabidopsis, which have defects in *AtPEX2* (*TED3*; (Hu et al., 2002)), *AtPEX5* (Zolman et al., 2000), *AtPEX10* (Sparkes et al., 2003), *AtPEX14* (Hayashi et al., 2000), and *AtPEX16* (*SSE1*; (Lin et al., 1999)).

5. Genetic approaches to understand plant peroxisomes

Recently, some studies of peroxisome defective mutants have been reported. They suggest that there are some other roles of plant peroxisomes in addition to β-oxidation, the glyoxylate cycle and glycolate pathway.

One of these mutants, *abnormal inflorescence meristem 1* (*aim1*), is defective in one of multifunctional proteins of β-oxidation in Arabidopsis (Richmond and Bleecker, 1999). After the transition to reproductive growth, the *aim1* inflorescence meristem becomes disorganized, producing abnormal floral meristems and resulting in plants with severely reduced fertility. The role of *AIM1* in determining the morphology of the reproductive organs is unclear. There are several possibilities but the most attractive

explanation is that *aim1* blocks the production of a lipid signaling molecule that is necerrary for intercellular communication in reproductive meristems (Richmond and Bleecker, 1999). The possible candidates for fatty acid-derived signal molecules are jasmonic acid, the traumatin family and related alkenals, highly oxygenated fatty acid derivatives and lipo-oligosaccharides.

Another example is *ted3* mutant, which have been identified as a dominant suppressor of *det1* phenotypes (Hu et al., 2002). Arabidopsis DET1 protein has been demonstrated to be a repressor of photomorphogenesis, since dark-grown *det1* mutant develops as light-grown plants, i.e. short hypocotyls, opened cotyledons with developed chloroplasts (Schwechjeimer and Deng, 2000). The *det1* mutant showed some features to suggest the peroxisomal activities were impaired (Hu et al., 2002). The *det1* mutant was developmentally arrested without supplemental sugar in dark grown seedlings. It also showed some degree of resistance to IBA that can be converted to toxic IAA in β-oxidation of peroxisomes. Electron micrographs showed that peroxisomes in light grown *det1-1* plants usually had a less dense matrix compared with wild type. Developmental defects and the abnormal expression of many genes in *det1* are rescued by *ted3*. The *TED3* encodes a 38-kD peroxisomal protein that is most similar to yeast and mammalian Pex2p, a *PEX2* gene product. The reason is beyond our present scope of plant peroxisomal functions. These mutants suggest that plant peroxisomes have some other roles that have not yet been identified.

6. Study for the genetic configuration of organelles

Recently, some groups have attempted to obtain comprehensive vision of genetic configuration of organelles, namely mitochondria and chloroplasts. One of the strategies is proteomic analyses and the other strategy is prediction of protein localization from the amino acid sequences. Novel proteins have been identified in those studies (Kruft et al., 2001; Millar et al., 2001; Koo and Ohlrogge, 2002; Peltier et al., 2002; Schubert et al., 2002). More recently, Mooth et al. (Mootha et al., 2003) performed to combine those two analyses to explore mitochondrial molecular composition, then advocated 591 mitochondrial proteins, including 163 proteins originated only in proteomic analysis and 192 proteins originated only in previous reports that those proteins associated with mitochondria. In terms of plant materials,

Arabidopsis is so suitable for these exhaustive analyses that it has been established the information of the complete genome sequences.

7. Aim of this study

Aims of this study are to reveal comprehensive vision of genetic configuration of plant peroxisomes, and to investigate functional diversity of plant peroxisomes. The bioinformatic analysis of the Arabidopsis genome is useful to survey peroxisomal proteins based on their PTS signals comprehensively. Based on the analysis, I performed microarray analysis to reveal differences of expression profiles in peroxisomes at various organs. In turn, to reveal genetic events during the process of the peroxisomal differentiation, I investigated gene expression profiles of germinating seedlings during illumination.

Chapter 1

Functional Differentiation of Peroxisomes Revealed by Expression Profiles of Peroxisomal Genes in *Arabidopsis thaliana*

1.1. Introduction

To understand full picture of functional differentiation in plant peroxisomes, I attempted to collect genes related peroxisomal function and biogenesis comprehensively. Differentiated peroxisomes that are glyoxysomes and leaf peroxisomes contain a unique set of enzymes that provide special functions in specific organs. For this reason, I attempt to categorize all candidates for peroxisomal genes by their expression profiles in various organs.

1.2. Materials and methods

Plant materials and growth conditions

Arabidopsis thaliana ecotype Columbia was used as the wild-type plant. All seeds were surface sterilized in 2% NaClO and 0.02% Triton X-100 and germinated on growth media containing 2.3 mg ml^{-1} MS salt (Wako, Osaka, Japan) 1% (w/v) sucrose, 100 μg ml^{-1} myo-inositol, 1 μg ml^{-1} thiamine-HCl, 0.5 μg ml^{-1} pyridoxine, 0.5 μg ml^{-1} nicotinic acid, 0.5 mg ml^{-1} MES-KOH (pH 5.7) and 0.8% agar. Seedlings were harvested after being grown for one day in dark conditions as seedlings, after one day in light conditions following growth for four days under dark conditions as cotyledons and for two weeks in light conditions as roots. For leaves (rosette leaves), stems, flowers and siliques (green siliques), seedlings grown for one week on the growth medium were transferred into a 1:1 mixture of perlite and vermiculite, and were then grown under a 16 h light (100 $\mu Em^{-2}s^{-1}$)/8 h dark cycle at 22°C for an additional four weeks.

Preparation of peroxisomal-specific DNA microarrays

Candidates of peroxisomal genes were selected based on their peroxisomal signal sequences (PTSs). In

this search, we used a pattern of PTS1, (C/ A/ S/ P)-(K/ R/ H)-(I/ L/ M), that is identified in Arabidopsis (Hayashi et al. 1997), although some different patterns of PTS1 were proposed by studies used other materials (Kragler et al. 1998, Mullen et al. 1997). PTS2 N-terminal presequences contained a (R)-(A/ L/ Q/ I)-X5-(H)-(L/ I/ F)

consensus sequence up to the 50th amino acid from the N-terminal, since the consensus sequence is found in N-terminal extra sequence (Kato et al., 2000). From these limitations, I have to mention that the peroxisomal gene candidates obtained by these search might contain omissions or have false inclusions. Peroxins and some proteins that did not include the PTSs, but were involved in peroxisomal function and biogenesis, were searched based on sequence similarity by a blast analysis to known genes in Arabidopsis, in other plants, in yeast or in mammals (Mullen et al. 2001).

Sequence similarity exceeding 30% identity and extending over at least 40 amino acids of the region were used as parameters to identify protein families in all peroxisomal genes by a BLASTP. Using the limiting value, all genes of each reported isogene were in a group and the group did not include the other genes.

Expressed sequence tag (EST) clones for some genes were obtained from the Kazusa DNA Research Institute or the Arabidopsis Biological Resource Center (ABRC). These ESTs were cloned in pBluescript SK-vector. To amplify the ESTs by PCR, we used the 5' primer 5'-AACAGCTATGACCATG-3' and the 3' primer 5'-GTAAAACGACGGCCAGTG-3'.

For clones amplified from genomic DNA, the DNA was extracted from rosette leaves using the Automatic Nucleic Acid Isolation System NA-2000 (KURABO, Osaka, Japan) according to protocols provided by the manufacturer. Each gene fragment was amplified by PCR using specific primer sets for each gene (see supplemental data for primer sequences). Upon request, all novel materials described in this publication will be made available in a timely manner for non-commercial research purposes.

DNA from the PCR reactions was purified by isopropanol precipitation and its quality and quantity was confirmed by electrophoresis in 1.0% agarose gel. The nucleotide sequence of all of the amplified DNA was determined to confirm that the correct fragments were amplified.

Microarrays were printed by TAKARA SHUZO CO., LTD and 269 DNA fragments spotted onto Takara Slides (Takara, Shiga, Japan) with the control genes, beta-6-tubulin, actin-2, glyceraldehyde-3-phosphate-dehydrogenase and Arabidopsis-chlorophyll-ab-binding-protein, with a negative control gene, pUC19, and with an external control gene, lambda-A. For details, see supplemental data.

RNA isolation and probe labeling

Total RNA was extracted from each tissue using the Concert™ Plant RNA Reagent (Invitrogen Corp., Carlsbad, CA, USA) according to protocols provided by the manufacturer. The total RNA was further purified by precipitation with LiCl. At least two independent RNA preparations for each biological sample were prepared and used for probe synthesis. Fluorescence-labeled probes were prepared from RNAs by the incorporation of fluorescent nucleotide analogs during first-strand reverse transcription. Each reaction (20 μL) consisted of 50 μg total RNA with 200 pg lambda poly $(A)^+$RNA-A (TX802; Takara, Shiga, Japan) as an external control, 300 pmol oligo-(dT) 18-mer primers, a dNTP mixture for Cy3 or Cy5 labeling, 40 units RNase Inhibitor, 400 units M-MLV Reverse Transcriptase in a 1x reaction buffer (TX810; Takara, Shiga, Japan) and 1 nmol of either Cy3-dUTP or Cy5-dUTP (Amersham Pharmacia Biotech, NJ, USA). RNA and primers were heated to 70°C (for 5 min) and cooled in ice before the remaining reaction components were added. The reverse transcription reaction proceeded for 1 hr at 42°C, followed by 10 min at 70°C to stop the reaction. To degrade the template, the components were incubated with 60 units RNaseH for 20 min at 37°C. The labeled cDNA was purified from the unincorporated dye molecules using a purification column (TX810; Takara, Shiga, Japan) and collected by ethanol precipitation following purification with a total volume of a chloroform/isoamyl alcohol mixture (24:1). The dried pellets were dissolved in 11 μl of hybridization buffer (6x SSC, 0.2% SDS, 5x Denhardt's solution, 0.1 mg ml^{-1} denatured salmon sperm DNA), which was filtered through a 0.22 μm filter, and denatured at 95°C before use in the hybridizations.

Microarray hybridization and data analysis

Hybridizations were performed for 16 hr at 65°C in humidified ArrayIt™ Hybridization Cassettes (TeleChem International, Inc., Sunnyvale, CA, USA). The slides were washed twice in 2x SSC with 0.2% SDS at 55°C for 30 min and once in 2x SSC with 0.2% SDS at 65°C for 5 min. Slides were then rinsed for 5 min in 0.05x SSC and dried by centrifugation. The fluorescent signatures were captured using a GMS 418 Array Scanner (Genetic MicroSystems, MA, USA) and analyzed using ImaGene™ 4.0 software (Biodiscovery, Los Angeles, CA, USA). The local background was subtracted from the value of each spot on the array and background fluorescence calculated based

on the fluorescence signal of the negative control gene, pUC. For analysis, only data with positive signal intensities that met the following three requirements were used: (1) the signal intensity was more than the local background plus twice that of the local background sample standard deviation (SD), (2) the signal intensity minus its local background was more than the pUC signal plus twice that of its local background SD, and (3) the signal intensity was less than 60000, the upper limit of the scanner. Normalization between the Cy3 and Cy5 fluorescent dye emission channels was achieved using the Log Linear Regression Method (refer to http://afgc.Stanford.edu/~finkel/talk.htm). I pooled four replicated data sets from two independent RNA preparations in each experiment, and then calculated its median. Gene clustering analysis was performed with GeneSpring software (Silicon Genetics, San Carlos, CA, USA).

Quantitative RT-PCR analysis

Total RNA from each organ of Arabidopsis was extracted using the same method as for the microarray (see above). The first strands from 5 μg total RNA in the 20 μL reaction mixture were synthesized using the SUPERSCRIPT™ First-Strand Synthesis system for RT-PCR (Invitorgen Corp., Carlsbad, CA, USA) according to the manufacturer's protocol using oligo(dT) primers. The PCR was performed using the SmartCycler Instrument (Cepheid, CA, USA) with the SYBR Green I (BioWhittaker Molecular Applications, ME, USA) and the Takara Ex Taq™ R-PCR Version kit for PCR (Takara, Shiga, Japan) according to the manufacturer's protocol. Each reaction was performed on 2 μL of a 10 times dilution of the first cDNA strands and synthesized as described above in a total reaction of 25 μL. Specific primer sets were designed for each gene (see supplemental data). The reactions were incubated at 95°C for 30 sec to activate the hot-start recombinant *Taq* DNA polymerase, followed by 40 cycles of 3 sec at 95°C and 20 sec at 68°C to measure the fluorescence signal. The specificity of the PCR amplification procedures was checked with a heat dissociation protocol (from 65°C to 95°C) after the final cycle of the PCR, and with a DNA gel electrophoresis analysis using a GeneGel Excel 12.5/24 kit (Amersham Biosciences, NJ, USA).

1.3. Results and discussion

Comprehensive survey of peroxisomal genes

At first, I collected genes of peroxisomal matrix proteins from the entire Arabidopsis genome sequences (see Materials and methods). The pattern search detected 182 gene candidates of PTS1-containing protein, which encoded proteins having a unique tripeptide sequence on the end of carboxyl-terminal; (C/ A/ S/ P)-(K/ R/ H)-(I/ L/ M) (Hayashi et al. 1997), and 74 gene candidates of PTS2-containing protein, which encoded proteins having an amino terminal presequence with a consensus sequence; (R)-(A/ L/ Q/ I)-X5-(H)-(L/ I/ F) (X stands for any amino acid) (Kato et al. 2000) (Fig. 1A). The functional identification status for the collected 256 gene candidates that encoded PTS1- and PTS2-containing proteins was carried out according to the short descriptions in the databases (TAIR: http://www.arabidopsis.org/). In the collected 256 genes, all genes that encode enzymes reported to function in peroxisomes were included. These included acyl-CoA oxidases, multifunctional proteins, 3-ketoacyl-CoA thiolases, citrate synthases, isocitrate lyase, malate synthase, malate dehydrogenases, glycolate oxidases, hydroxypyruvate reductase, serine-glyoxylate aminotransferase and glutamate-glyoxylate aminotransferases.

In the second step, I collected proteins that did not have any obvious peroxisomal targeting signals, but were related to peroxisomal functions or biogenesis (see Materials and methods). One of these proteins was catalase, which is well known to be localized in peroxisomes, but had no obvious PTS1 or PTS2 signal (Beevers, 1979; Kamigaki et al., 2003). Another protein was cytosolic aconitase, which is responsible for the glyoxylate cycle that is well-known as a peroxisomal function (Hayashi et al., 1995). The catalase and aconitase genes were searched using the keywords "catalase" or "aconitase". Results indicated that there were three genes each of catalase and aconitase in the entire Arabidopsis genome (Fig. 1A).

The targeting signals for peroxisomal membrane proteins have not been identified. We investigated the list of Arabidopsis_thaliana_MatDB in the PEDANT Web server using the keyword "peroxisome" and collected seven genes of membrane proteins that were annotated as peroxisomal, but did not have any PTS signals (Fig. 1A). These included two genes that have been reported to be peroxisomal, a peroxisomal

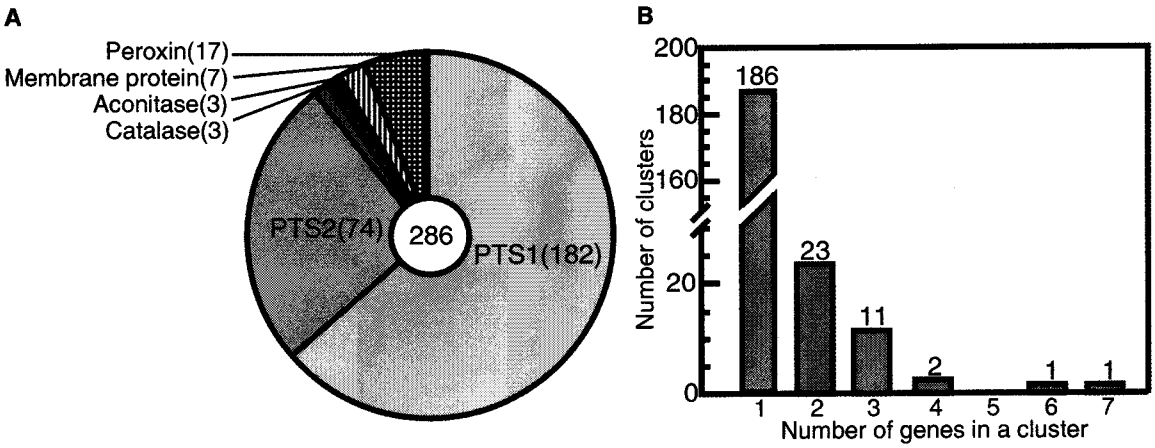

Fig. 1 Comprehensive survey of peroxisomal genes. (A) Genes that encoded peroxisomal proteins were predicted from ORF protein sequences of the entire *Arabidopsis* genomic sequence on the PEDANT Web server (http://pedant.gsf.de/). The total number of peroxisomal genes is in the center of circle. Numbers in parentheses represent the number of genes in each category.
(B) Amino acid sequence similarity of the 286 gene products were compared using the BLASTP program.

ABC transporter (PED3) and an L-ascorbate peroxidase (Yamaguchi et al., 1995; Hayashi et al., 2002b). I then used a keyword search for "peroxins", which are related in peroxisomal biogenesis, and found 17 gene candidates that encoded peroxins based on reported sequences of peroxins in other organisms (Fig. 1A) (see supplemental data) (Mullen et al., 2001).

With the addition of these genes for non-PTS1- and PTS2-containing proteins, I obtained 286 gene candidates that encoded proteins related in peroxisomal function and biogenesis in Arabidopsis (Fig. 1A). A BLASTP analysis using the amino acid sequences of the 286 peroxisomal genes divided them into 186 single genes and 38 gene families (Fig. 1B), of which the largest family consisted of seven genes that encoded the 4-coumarate-CoA ligase family. It was suggested that there were at least 224 kinds of functional proteins with amino acid sequence similarity in plant peroxisomes, although only 29 of them have been experimentally determined to function in peroxisomes of higher plants (see supplemental data). This suggests the existence of more diverse functions in plant peroxisomes than previously known.

Construction of a peroxisome-specific DNA microarray

To reveal the functional diversity of peroxisomes in higher plants, I applied a microarray analysis and classified the peroxisomal genes by their expression profiles in Arabidopsis organs. I attempted to amplify fragments of peroxisomal genes from cDNA clones (151 clones) or genomic DNA (135 genes) to construct a microarray that covered all gene-encoding candidates of peroxisomal proteins (see supplemental data). The amplified DNA fragments were confirmed by determination of their sequences. Finally, 263 genes of total 286 peroxisomal genes were arrayed on a slide, because 23 genes were amplified in a mixture of some distinct products or in no products. The detailed construction of the peroxisomal microarray is described in the supplemental data.

Expression profiles of peroxisomal genes

Using the peroxisomal microarray, I examined gene expression profiles in seven organs: seedlings, cotyledons, leaves, stems, flowers, siliques and roots (see Materials and methods). Because the genes expressed in glyoxysomes and leaf peroxisomes are well documented in seedlings and cotyledons, respectively, they are useful in confirming the appropriateness of the results from our microarray assay and the

following clustering analyses. The gene expression profile in each organ was compared on an array with a control that was a mixture of equal volumes of total RNA from all seven organs. This comparison tends to select genes that are strongly expressed in one or two organs preferentially, because genes showed small differences between organs were averaged in the control sample.

To minimize the inherent variability of the microarray assay and to ensure the reliability of our results, at least four replicates with two independent RNA preparations were used to analyze the mRNA abundance of each sample pair. Each hybridization signal was normalized using a log linear regression method (refer to http://afgc.Stanford.edu/~finkel/talk.htm) and Cy3/Cy5 signal ratios of four replicates were calculated to give median values. A comparison of the gene expression profiles from Cy3- and Cy5-labeled control samples co-hybridized on an array shows that all data points varied within a two-fold range, which demonstrates a high reproducibility of the procedure (Fig. 2A). An averaged Cy3/Cy5 signal ratio of lambda DNA as an external control and tubulin or actin as internal controls was found within two-fold change lines in every experiment (Fig. 2A-H).

Classification of plant peroxisomes by gene expression profiles in seven Arabidopsis organs

A clustering analysis gives a holistic view because co-expressed genes in specific organs provide clues regarding the functional features of the different peroxisomes in each organ. I used 125 peroxisomal genes, which showed positive signal intensities in the control samples of all organs examined (see Materials and methods), for a K-mean clustering analysis using GeneSpring software. I divided them into six groups (Fig. 3A-F).

Primary set of components in plant peroxisomes

Group A contains 68 genes that were expressed at similar levels in all organs examined (Table 1). Up to the present, only catalase is definitely known to exist ubiquitously in plant peroxisomes (Beevers, 1979). The fact that there are so many genes expressed in all organs suggests that plant peroxisomes have many more basic functions than we are aware of at present. The genes in group A can be classified into five subgroups by their predicted functions.

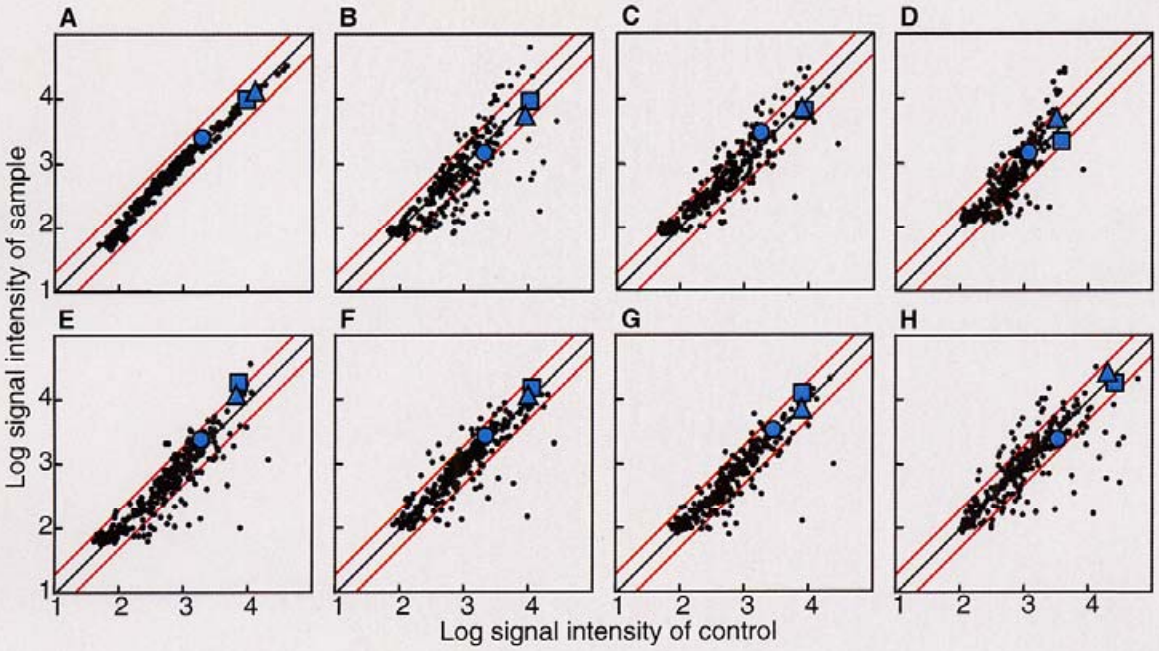

Fig. 2 Expression of putative peroxisomal genes in various organs. Scatter plots indicate signal intensities of the peroxisomal genes in hybridizations with sample and control probes. The y-axis represents log10 (signal intensity of sample), while the x-axis represents the control; a mixture of the total RNAs from each organ in equal volumes. RNAs used as samples in the panels are: (A) control, (B) seedling, (C) cotyledon, (D) leaf, (E) stem, (F) flower, (G) silique and (H) root. The black line represents the regression line, while red lines represent signal ratios of 2.0 and 0.5. Blue circles represent lambda DNA as an external control. Blue triangles and blue squares represent actin and tubulin as internal controls, respectively.

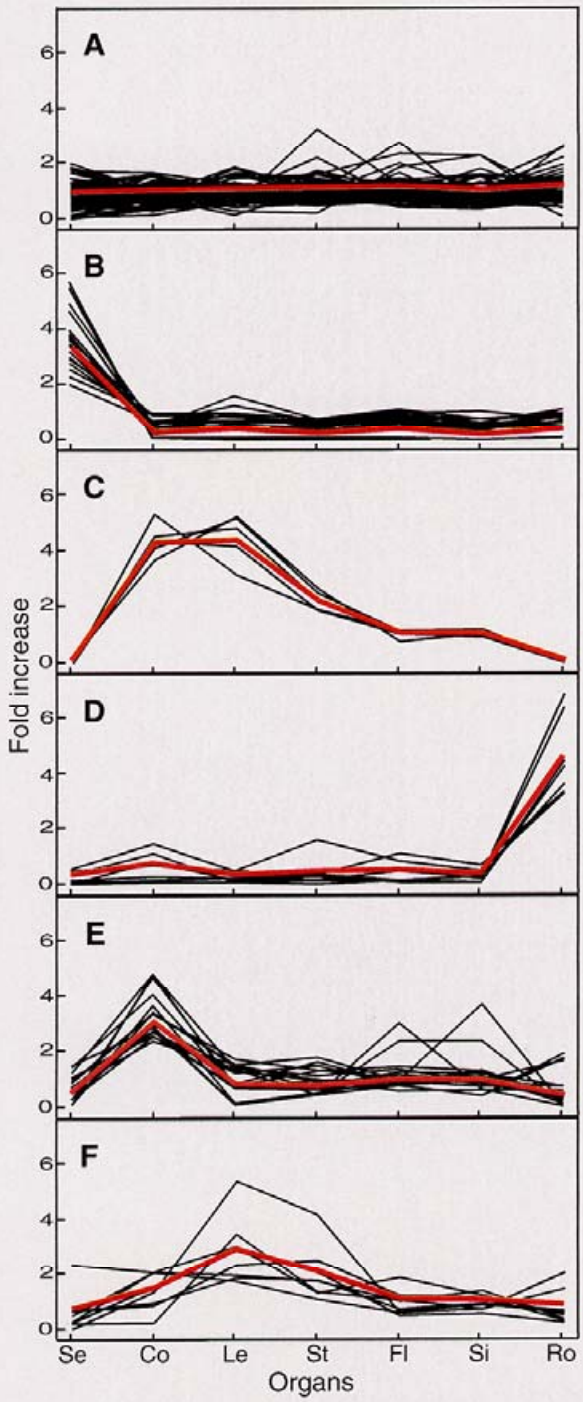

Fig. 3 Classification of putative peroxisomal genes by organ-dependent expression profiles. Fold increases of a peroxisomal gene is defined as a median of the ratio (signal intensity of sample / signal intensity of control) obtained from four repeated hybridizations. A K-mean clustering analysis of the data indicated that most of the peroxisomal genes could be classified into six groups, (A-F), in terms of organ-dependent gene expression. One lateral line in each panel represents the organ-dependent expression of one peroxisomal gene. The x-axis represents the organs examined: (Se) seedling, (Co) cotyledon, (Le) leaf, (St) stem, (Fl) flower, (Si) silique and (Ro) root. The y-axis represents the fold increase of a gene in each organ and the red line indicates the mean of the fold change of all genes classified into each group. Data were analyzed using GeneSpring software.

Table 1 Genes Classified into Group A

Gene ID	Annotation	Relative Expression Levels [a]							Peroxisomal
		Seedling	Cotyledon	Leaf	Stem	Flower	Silique	Root	Targeting Signal
β-Oxidation and Related Fatty Acid Metabolism									
At4g39850	PED3	1.68	0.96	1.19	1.14	1.22	0.77	0.86	-[b]
At1g18460	Lipase family	0.91	1.80	1.23	1.60	0.87	0.88	1.12	PTS1
At5g16370	Acyl-CoA synthetase	1.33	1.07	2.13	1.27	0.85	1.04	0.85	PTS1
At4g16760	ACX1	1.24	0.98	1.46	1.02	1.72	1.17	1.77	PTS1
At2g35690	Acyl-CoA oxidase putative	1.03	0.90	1.22	1.29	1.41	1.22	1.41	PTS1
At3g51840	ACX4	2.07	0.86	1.47	1.40	0.99	0.83	0.68	PTS1
At4g05530	Short-chain dehydrogenase/ reductase family protein	1.58	0.67	1.39	0.98	1.37	1.10	1.49	PTS1
At4g29010	AIM1	0.75	1.12	1.81	1.17	2.29	1.29	1.03	PTS1
At5g48880	KAT5	0.39	1.04	0.54	0.48	2.15	2.54	0.59	PTS2
At1g01710	Acyl-CoA thioesterase, putative	0.64	1.33	1.16	1.06	1.08	1.13	0.98	PTS1
At1g48320	Thioesterase family	0.39	1.00	1.12	1.88	0.77	1.75	1.70	PTS1
At3g61200	Thioesterase-related	0.34	0.91	1.71	1.86	1.15	1.28	1.58	PTS1
Blanched Chain Amino Acid Degradation									
At2g26800	Hydroxymethylglutaryl-CoA lyase putative	0.36	1.06	1.14	2.44	0.93	1.56	0.89	PTS1
At3g15290	3-Hydroxybutyryl-CoA dehydrogenase putative	1.20	1.00	1.08	0.94	1.27	1.15	1.36	PTS1
At5g65940	CHY1	1.26	1.17	1.24	1.00	1.34	1.23	1.04	PTS1
At5g43280	Enoyl-CoA hydratase-like protein	1.11	1.03	0.96	1.02	1.30	1.49	1.37	PTS1
Glyoxylate Cycle									
At3g58750	Citrate synthase -like protein	1.71	0.70	0.78	0.92	1.19	1.24	0.98	PTS2
At4g26970	Putative aconitase	1.20	0.82	1.53	0.88	1.16	0.64	2.87	-
Peroxin									
At1g48640	PEX-3 like protein	1.55	1.12	0.86	0.83	1.07	0.74	1.04	-
At5g25760	PEX-4 like protein	0.81	0.97	1.19	1.44	1.39	1.15	1.63	-
At5g56290	PEX-5	2.04	1.11	1.23	0.89	1.14	0.89	0.87	-
At3g07560	PEX-13 like protein	1.48	0.93	1.90	1.70	1.18	0.83	1.32	-
At5g62810	PEX-14	1.68	1.26	1.37	0.97	1.08	0.83	0.68	-
At3g03490	PEX-19 like protein	1.08	0.83	1.02	1.14	0.92	0.97	0.93	-
Degradation of Hydrogen Peroxide									
At3g52880	Monodehydroascorbate reductase (NADH) - like protein	0.98	1.57	1.75	1.67	1.17	1.15	0.96	PTS1
At4g35000	L-ascorbate peroxidase	1.99	1.94	1.49	1.22	1.39	0.93	0.66	-
At1g54340	NADP specific isocitrate dehydrogenase	1.27	0.90	1.20	0.89	1.14	1.06	2.02	PTS1
At3g02360	6-Phosphogluconate dehydrogenase-related	0.74	0.68	0.92	2.01	1.30	1.26	2.20	PTS1
Others									
At1g20540	Transducin / WD-40 repeat protein family	0.72	1.44	0.86	1.08	1.24	1.29	1.06	PTS1
At1g24530	Transducin / WD-40 repeat protein family	0.58	1.56	0.66	1.42	0.68	0.56	1.45	PTS2
At1g53850	20S proteasome alpha subunit E1	2.09	1.26	0.79	0.95	1.28	1.25	1.36	PTS1
At1g77440	20S proteasome beta subunit PBC2 putative	1.88	1.22	0.68	0.81	1.30	1.15	1.44	PTS2
At2g06050	12-Oxophytodienoate-10,11-reductase	0.35	0.68	2.11	0.71	1.94	0.61	1.80	PTS1
At2g11900	Pseudogene	1.52	1.82	0.82	0.91	0.71	0.95	0.65	PTS2
At2g25730	Expressed protein	0.98	1.41	1.10	0.84	0.85	0.83	1.08	PTS2
At2g26230	Putative uricase subunit	0.78	0.68	1.34	1.26	1.30	1.05	1.69	PTS1
At2g27890	Hypothetical protein	0.82	0.94	0.98	1.16	1.20	1.14	1.33	PTS1
At2g38400	Beta-alanine-pyruvate aminotransferase putative	1.01	0.53	1.05	1.22	3.05	0.75	0.86	PTS1
At2g41790	Putative zinc protease	1.32	1.20	1.25	1.22	0.96	0.80	1.17	PTS1

At2g42490	Putative copper amine oxidase	0.53	1.11	1.08	1.55	1.18	1.07	1.94	PTS1
At2g43020	Putative amine oxidase	0.30	0.40	0.83	1.80	2.60	2.56	1.00	PTS1
At2g46020	Putative SNF2 subfamily transcriptional activator	1.05	0.89	1.12	0.95	0.86	0.69	0.89	PTS1
At3g06810	Acetyl-CoA dehydrogenase putative	1.02	1.33	1.21	0.94	1.11	0.84	1.06	PTS1
At3g12790	Unknown protein	0.88	1.25	1.48	1.17	1.64	1.81	1.55	PTS1
At3g17020	Expressed protein	0.80	0.87	2.03	1.33	1.67	1.25	2.43	PTS1
At3g48170	Betaine aldehyde dehydrogenase-like protein	1.15	1.29	0.94	1.01	0.69	0.92	1.34	PTS1
At3g56460	Quinone reductase-like protein	1.13	1.44	1.41	1.21	1.30	1.52	1.72	PTS1
At3g59050	Similar to polyamine oxidase	0.35	0.91	0.90	1.61	1.14	2.02	0.98	PTS1
At3g61960	Serine/threonine-protein kinase-like protein	1.70	0.72	0.88	0.72	0.84	0.59	0.92	PTS1
At4g01570	Pentatricopeptide (PPR) repeat-containing protein	0.79	1.55	1.02	0.99	1.17	1.04	1.57	PTS1
At4g04470	PMP22	0.60	0.71	1.26	0.90	1.60	0.89	0.89	-
At4g05160	4-Coumarate-CoA ligase like protein	1.19	1.48	1.11	1.24	0.72	0.74	1.05	PTS1
At4g15330	Cytochrome P450 like protein	0.75	1.66	1.34	1.16	0.93	0.92	1.39	PTS1
At4g24550	Clathrin coat assembly like protein	1.09	1.06	1.06	1.19	1.38	1.30	1.77	PTS1
At4g27760	Forever young gene	1.44	1.51	0.77	0.87	1.49	1.23	1.16	PTS1
At4g39140	Expressed protein	0.58	0.63	1.92	1.28	1.27	0.88	1.45	PTS1
At4g39660	Alanin aminotransferase	1.01	0.96	0.89	1.83	1.50	1.50	1.47	PTS1
At4g39880	Expressed protein	1.42	1.80	0.68	0.80	1.15	0.98	0.81	PTS2
At5g02030	Putative homeodomain protein	0.26	0.61	0.74	3.53	1.48	1.75	0.31	PTS2
At5g09330	Expressed protein	2.23	1.24	1.01	0.90	1.43	0.93	1.62	PTS1
At5g13070	Expressed protein	0.88	1.56	1.35	0.99	1.37	0.91	1.39	PTS1
At5g18830	Squamosa promoter binding protein-like 7	0.71	1.62	0.86	1.50	1.14	1.24	1.24	PTS2
At5g23050	Acetyl-CoA synthetase-like protein	0.54	1.81	0.80	0.82	0.88	0.92	1.51	PTS1
At5g24400	6-Phosphogluconolactonase-like protein	0.72	1.36	0.78	0.98	1.57	1.52	2.85	PTS1
At5g40830	Expressed protein	1.00	0.97	0.40	1.31	1.06	1.76	1.12	PTS1
At5g41210	Glutathione transferase	0.39	0.59	1.76	1.61	1.40	1.19	1.80	PTS1
At5g42620	Major surface glycoprotein-like	0.76	1.44	0.86	1.04	0.91	0.94	0.82	PTS1
At5g44250	Expressed protein	1.71	1.15	0.99	0.88	1.06	1.03	0.90	PTS1

[a] Ratio of signal intensity of RNA from each organ to that of RNA mixture from seven organs as a control.

[b] A gene whose product does not have obvious peroxisomal targeting signals.

The first sub-group consists of 12 genes that function in β-oxidation and coincide with a previous report that the β-oxidation pathway was shown to be ubiquitous in plant cells (Table 1) (Gerhardt, 1983). There is an entire set of genes in the β-oxidation pathway: a medium- to long-chain acyl-CoA oxidase (ACX1) (Hooks et al., 1999), a short-chain acyl-CoA oxidase (ACX4) (Hayashi et al., 1999), a multifunctional protein (AIM1) (Richmond and Bleecker, 1999) and a 3-ketoacyl-CoA thiolase (KAT5) (Germain et al., 2001). It should be mentioned that the set of genes for β-oxidation is different from the glyoxysomal set classified into group B. In addition to the genes that encode core β-oxidation enzymes, this group also includes a gene (At4g05530) that is 46% identical to the human peroxisomal 2,4-dienoyl-CoA reductase involved in the degradation of unsaturated fatty acids (Kindl, 1993).

The second sub-group consists of four genes that encode proteins for the degradation of the blanched chain amino acids (BCAAs): valine, leucine and isoleucine (Table 1). Degradation of BCAAs is a peripheral pathway of β-oxidation and the subcellular location of BCAA catabolism in plants remains controversial (Hooks 2002). Although BCAA catabolism occurs in the mitochondria in mammals, it has been reported that peroxisomes isolated from mung bean and sunflower cotyledons convert labeled BCAAs to propionyl-CoA and acetyl-CoA (Gerbling and Gerhardt, 1988, 1989). The involvement of 3-hydroxyisobutyryl-CoA hydrolase in BCAA catabolism was revealed by a genetic screening to isolate mutants, called *chy1*, which were disrupted in the conversion of indole-3-butyric acid (IBA) to indole-3-acetic acid (IAA) (Zolman et al., 2001). The result of our microarray analysis showed that the genes involved in the degradation of BCAAs, which included the CHY1, were expressed in every organ and suggested that plant peroxisomes act to break down BCAAs as a basic function.

The third sub-group consists of two genes that encode a citrate synthase-like protein and a putative aconitase (Table 1). Because the other enzymes of the glyoxylate cycle in peroxisomes are not found in group A, the citrate synthase-like protein (At3g58750) and putative aconitase (At4g26970) do not function as members of the glyoxylate cycle, but might be involved in other metabolic function. Our microarray data dearly showed that these citrate synthase and aconitase genes were not expressed markedly in seedlings.

The fourth sub-group consists of six genes that encode peroxins identified as peroxisome biogenesis factors (Table 1). The microarray data suggested that these

peroxins exist in all organs to maintain functional peroxisomes. Among the peroxins, only PEX14, PEX5 and PEX7 were reported to function in higher plants (Nito et al., 2002). Pex14p is predicted to play a role for peroxisome protein import of both PTS1- and PTS2-containing proteins on the peroxisomal membrane, and Pex5p and Pex7p are predicted to play a role for PTS1 and PTS2 receptors in the cytosol, respectively (Nito et al., 2002). In the microarray analysis, these peroxins were expressed in all organs examined, except for the case of PEX7 in flowers (Table 1, data of PEX7 was excluded from the clustering analysis). In terms of genes that encode the cargo proteins of Pex5p and Pex7p, that is the PTS1- and PTS2-containing proteins, respectively, genes of both cargo proteins were expressed in all organs examined, although the number of genes that encoded the PTS2-containing protein were less than those of the PTS1-containing protein. These data suggested that PTS2-containing proteins exist in all plant peroxisomes, although most PTS2-containing proteins up to now are known as glyoxysomal (Kato et al., 1995; Kato et al., 1996; Hayashi et al., 1998a; Kato et al., 1998). Except for these genes of reported PTS2-containing proteins, sixty-one genes of total 74 genes for PTS2-containing proteins are unidentified its function in peroxisomes, which suggests that they play a role in unidentified metabolic pathways of plant peroxisomes.

The fifth sub-group consists of four genes that encode proteins related to the degradation of H_2O_2 (Table 1). Monodehydroascorbate reductase (At3g52880) and ascorbate peroxidase (At4g35000) participate in the ascorbate-glutathione cycle for H_2O_2 removal (Jimenez et al., 1997; Corpas et al., 2001). Because this cycle requires NADPH as a cofactor, NADP specific isocitrate dehydrogenase (At1g54340) and 6-phosphogluconate dehydrogenase (At3g02360) in this sub-group might be responsible for accomplishing the formation of NADPH by a reduction of $NADP^+$. Although dehydroascorbate reductase and glutathione reductase are also needed for this cycle, genes encoding these enzymes were not identified in our search for peroxisomal genes. It is considered that candidates for those genes are annotated as unknown. Within the plant cell, catalase is located in the peroxisomes and is known as an enzyme for scavenging H_2O_2 (Beevers, 1979). In our microarray analysis, all three catalase genes in Arabidopsis were expressed in all organs but divided into group F because of a higher expression in the leaves and/or stems than in other organs. It is reported that the K_m of ascorbate peroxidase for H_2O_2 is much lower than that of catalase (Chen and Asada,

1989; Koshiba, 1993). These results suggest that H_2O_2 scavenging is performed by the cooperative efforts of catalase and the ascorbate-glutathione cycle, and is a constitutive function of plant peroxisomes.

Unlike existing reports, the microarray analysis revealed that plant peroxisomes, even glyoxysomes and leaf peroxisomes, have constitutive functions such as β-oxidation, BCAA catabolism, degradation of H_2O_2 and some unidentified functions that involve many more genes than I am aware of at present.

Diversity of plant peroxisomes by organ dependent gene expression profiles

In addition to the primary set of peroxisomal genes (Fig. 3A), the K-mean clustering analysis showed four groups of genes that have different expression patterns depended on organs (Fig. 3B-E) and a group of rest genes that did not have a clear expression pattern in common (Fig. 3F). The analysis suggested that plant peroxisomes are divided into at least four groups that have a set of genes expressed in special organs in addition to the primary set of genes.

Glyoxysomes

Group B is characterized by genes that showed a high expression in seedlings and a low expression in other organs (Fig. 3B, Table 2). The seedlings included another entire set of genes that encoded enzymes of β-oxidation and the glyoxylate cycle, suggesting that they possessed glyoxysomes, which is consistent with previous studies (Beevers, 1979). Because two acyl-CoA oxidases, that are ACX1 and ACX4, were ubiquitously expressed in seedlings (Table 1), glyoxysomes are capable of performing the complete β-oxidation of triacylglycerols. Two other acyl-CoA oxidases, ACX2 and ACX3, which are specific for long-chain acyl-CoAs (Hooks et al., 1999) and medium-chain acyl-CoAs (Eastmond et al., 2000b; Froman et al., 2000), respectively, were divided into group B (Table 2). These results indicate that glyoxysomes in seedlings are specialized in the prompt utilization of fatty acids of storage lipids during post-germinative growth.

Additionally, six genes showed similar expression patterns with the genes of glyoxysomal enzymes. It was suggested that these genes function in concert with β-oxidation and the glyoxylate cycle in peroxisomes. One of these genes encodes an aspartate aminotransferase, Asp3 (Schultz and Coruzzi, 1995). An aspartate

Table 2 Genes Classified into Group B

Gene ID	Annotation	Relative Expression Levels [a]							Peroxisomal
		Seedling	Cotyledon	Leaf	Stem	Flower	Silique	Root	Targeting Signal
β-Oxidation									
At3g06860	AtMFP2	4.69	0.38	0.56	0.43	0.77	0.44	0.66	PTS1
At2g33150	PED1/KAT2	4.01	0.52	1.62	0.81	1.05	0.63	0.67	PTS2
At5g65110	ACX2	3.69	0.68	0.72	0.59	0.71	0.38	0.69	PTS2
At3g05970	AtLACS6	3.49	0.75	0.91	0.68	0.63	0.63	1.11	PTS2
At1g04710	KAT1	3.48	0.66	1.26	0.72	0.89	0.68	0.62	PTS2
At5g27600	AtLACS7	3.21	0.44	0.71	0.57	0.58	0.48	0.91	PTS2
At1g06310	Acyl-CoA oxidase-like	2.99	0.67	0.64	0.65	0.66	0.44	0.86	PTS2
At1g06290	ACX3	2.80	0.72	0.67	0.72	0.86	0.42	0.86	PTS2
Glyoxylate Cycle									
At5g03860	Malate synthase-like protein	5.77	0.06	0.04	0.03	0.03	0.02	0.01	PTS1
At3g21720	Putative isocitrate lyase	5.56	0.12	0.08	0.06	0.12	0.04	0.06	PTS1
At2g05710	Cytoplasmic aconitate hydratase	3.80	0.55	0.94	0.47	0.89	0.41	1.13	- [b]
At2g22780	Putative glyoxysomal malate dehydrogenase precursor	3.67	0.90	0.88	0.69	0.67	0.55	0.88	PTS2
At3g58740	Citrate synthase-like protein	2.05	0.69	0.69	0.81	1.00	1.06	0.78	PTS2
Others									
At5g11520	Asp3	4.98	0.62	0.42	0.40	0.60	0.43	0.54	PTS2
At5g42890	Expressed protein	4.67	0.45	0.40	0.51	0.66	0.64	0.54	PTS1
At4g21490	NADH dehydrogenase-like protein	3.85	0.23	0.43	0.36	0.59	0.30	0.97	PTS1
At1g49670	Putative ARP protein	2.94	0.94	0.84	0.62	0.87	0.81	1.00	PTS1
At2g39970	PMP38	2.63	0.91	0.99	0.82	1.17	0.81	0.95	-
At5g08470	PEX1-like protein	2.34	0.98	1.02	0.78	0.96	0.79	0.84	-

[a] Ratio of signal intensity of RNA from each organ to that of RNA mixture from seven organs as a control.

[b] A gene whose product does not have obvious peroxisomal targeting signals.

aminotransferase, together with malate dehydrogenase, has been suggested to form an electron shuttle system for the reoxidation of NADH generated in the glyoxysomes (Huang et al., 1983). Asp3 might correspond to the aspartate aminotransferase. PMP38 was identified with a peroxisomal membrane protein that has the energy-transfer-protein signature conserved residue characteristic of peroxisomal ATP/ADP carrier protein homologues (Fukao et al., 2001). Generally, the mitochondrial ATP/ADP carrier protein exchanges ATP synthesized in the matrix with ADP in the cytosol. In the glyoxysomes, triacylglycerols are firstly activated to fatty acyl-CoA by a fatty acyl-CoA synthetase in the presence of CoA, $MgCl_2$ and ATP (Cooper, 1971). Since ATP-synthesis pathways have not been found in the peroxisomes, PMP38 seems to import ATP into the peroxisomes.

Isogenes of enzymes for β-oxidation and the glyoxylate cycle

The alignment analysis showed that some glyoxysomal enzymes are coded with two or three genes (see supplemental data). To compare the expression pattern of these homologues in each gene family, I determined the amounts of their mRNA by quantitative RT-PCR in each organ (Fig. 4).

In terms of genes that encode the β-oxidation enzymes, the microarray data and quantitative RT-PCR data suggest that they could be divided into two groups: a glyoxysome-specific group and a constitutive group (Table 1, 2, Fig. 4A-D). In glyoxysomes, ACX2 and ACX3 function to degrade long- and medium-chain acyl-CoA, respectively (Table 2, Fig. 4B), and ACX1 constantly functions instead of them (Fig. 4A). The homologues of ACX1 (At2g35690) and ACX3 (At1g06310) were detectable in a quantitative RT-PCR analysis, but at very low levels (Fig. 4A-B). They might be expressed in a specific condition that I have not examined. As for the genes of the multifunctional proteins, AtMFP2 is glyoxysomal and AIM1 is constitutive (Fig. 4C). These results are consistent with the reports of Richmond and Bleecker (1999) and Eastmond and Graham (2000). With respect to genes of the 3-ketoacyl-CoA thiolases, PED1/KAT2 showed high amount expression in glyoxysomes, and both PED1/KAT2 and KAT1 existed constitutively, which suggested that PED1/KAT2 was a major gene of 3-ketoacyl-CoA thiolase (Fig. 4D). In contrast, KAT5 was predominantly expressed in flowers and siliques (Fig. 4D) and might be considered to have different substrate specificity to PED1/KAT2 and KAT1.

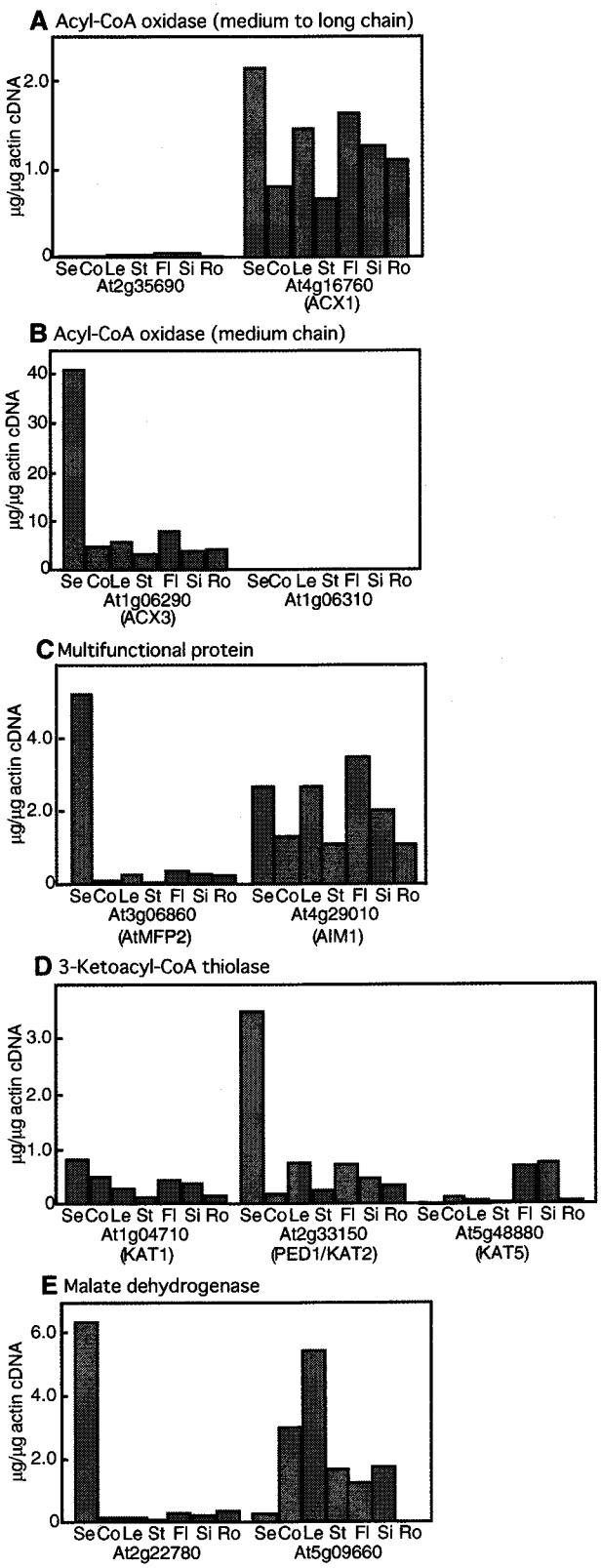

Fig. 4 Organ-dependent expression of genes that encode proteins with sequence similarity one another. The expression of genes belonging to some gene families were examined using quantitative RT-PCR. The gene families examined were: (A) medium- and long-chain acyl-CoA oxidase, (B) medium-chain acyl-CoA oxidase, (C) multifunctional protein, (D) 3-ketoacyl-CoA thiolase and (E) malate dehydrogenase. The y-axis represents the amount of transcript, while the x-axis represents the organs examined: (Se) seedling, (Co) cotyledon, (Le) leaf, (St) stem, (Fl) flower, (Si) silique and (Ro) root.

Malate dehydrogenase has been suggested to exist and to form an electron shuttle system for the reoxidation of NADH generated in both the glyoxysomes and leaf peroxisomes (Huang et al., 1983). The results of quantitative RT-PCR revealed that At2g22780 is glyoxysomal and At5g09660 is leaf peroxisomal (Fig. 4E).

These data indicate that a set of genes expressed specifically in seedlings make peroxisomes in seedlings categorize as glyoxysomes.

Thus, differences of transcription level and expression pattern in each organ between these isogenes were showed by quantitative RT-PCR analyses (Fig. 4). In the microarray analyses, these isogenes were divided into different groups (Table 1, 2 and 3) and it coincided with the results by quantitative RT-PCR. Therefore, expression profiles of these isogenes did not seem to reflect the effect of cross-hybridization in the microarray analyses.

Leaf Peroxisomes

Group C genes are expressed in both green cotyledons and rosette leaves (Fig. 3C, Table 3) and they consist of five genes reported as leaf peroxisomal enzymes except for genes of glycolate oxidase. This suggests that the green cotyledons and rosette leaves have so-called leaf peroxisomes. In the Arabidopsis genome, three genes of glycolate oxidase (At3g14415, At3g14420 and At4g18360) were predicted, but two of the three genes, At3g14420 and At4g18360, did not be amplified by PCR as a single band when the microarray was constructed. Therefore only At3g14415 gene was plotted on the arrays, which was categorized in group F (Fig. 3). Therefore, I characterized expression profiles of three glycolate oxidase genes by quantitative RT-PCR with isoform-specific primers. At3g14420 showed the highest expression level and its pattern agreed with that of the leaf peroxisomal gene (Fig. 5). As such, the At3g14420 gene could clearly be included in group C, which indicated that all genes for glycolate metabolism could also be included.

In our microarray study, each set of genes that functioned as glyoxysomes or leaf peroxisomes were placed into one group (groups B and C, respectively) by their expression pattern. These results agree with previous studies (Beevers 1979) and indicate that our microarray data and clustering analyses were highly reliable in categorizing functional sets of peroxisomal genes. Therefore, our data could also be used to reliably categorize other functionally specialized plant peroxisomes.

Table 3 Genes Classified into Group C

Gene ID	Annotation	Relative Expression Levels [a]							Peroxisomal
		Seedling	Cotyledon	Leaf	Stem	Flower	Silique	Root	Targeting Signal
Glycolate Metabolism									
At5g09660	Malate dehydrogenase NAD-dependent	0.21	3.65	5.21	2.67	0.79	1.09	0.08	PTS2
At1g68010	Hydroxypyruvate reductase	0.05	5.30	3.16	1.91	1.18	1.20	0.05	PTS1
At1g70580	AtGGT2	0.06	4.50	4.80	2.25	1.18	1.12	0.14	PTS1
At1g23310	AtGGT1	0.06	4.08	5.13	2.49	1.13	1.06	0.13	PTS1
At2g13360	AGT1	0.01	4.35	4.14	1.88	1.05	0.96	0.03	PTS1

[a] Ratio of signal intensity of RNA from each organ to that of RNA mixture from seven organs as a control.

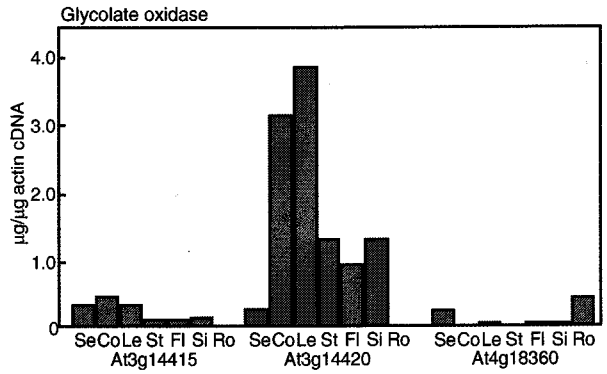

Fig. 5 Quantitative RT-PCR for transcripts of glycolate oxidase genes. Organ-dependent expression of three genes encoding glycolate oxidase was determined by quantitative RT-PCR. The y-axis represents the amount of transcript, while the x-axis represents the organs examined: (Se) seedling, (Co) cotyledon, (Le) leaf, (St) stem, (Fl) flower, (Si) silique and (Ro) root.

Peroxisomes in roots

Group D comprised genes that were especially expressed in roots (Fig. 3D). Although peroxisomes in roots have been categorized as unspecialized peroxisomes whose function is unknown to date, our microarray analysis revealed that there is a group of peroxisomal genes specifically expressed in the roots. This suggests that peroxisomes in roots are different and contribute to some root-specific functions. I collected genes that showed the signal intensity was at least three times higher than those of the control only in roots from the microarray analysis, and the expression profiles of these genes were confirmed by quantitative RT-PCR analysis (Table 4, Fig. 6). The genes especially expressed in the roots are not included in genes that function in established metabolic pathways such as glyoxysomes and leaf peroxisomes. In previous studies, polyamine oxidases have been reported to be located in peroxisomes of both mammalian and yeast cells (Table 4) (Nishikawa et al., 2000; Wu et al., 2003). In yeast *Candida. boidinii*, it was suggested that both peroxisomal polyamine oxidase and copper-containing methylamine oxidase play distinct roles in the utilization of nitrogen sources (Nishikawa et al., 2000). In plant roots, peroxisome may have similar function in terms of the utilization of nitrogen sources.

Peroxisomes in green cotyledons

Group E consisted of a group of genes especially expressed in green cotyledons (Fig. 3E). Based on these microarray analyses, I listed the genes that showed specific expression in green cotyledons that had a signal ratio greater than three, as same as those in Table 4 (Table 5). These genes were distinguished from the group of leaf peroxisomal genes (Fig. 3C) because they were only slightly expressed in rosette leaves (Fig. 3E). The function of all genes in this group has not been experimentally identified. Two methyltransferases, At2g43910 and At2g43920, are included in this group. Methyltransferases are suggested to be involved in the detoxification of reactive thiols that are produced upon glucosinolate degradation in *Brassica oleracea* L. (Attieh et al., 2000). Peroxisomes in green cotyledons of Arabidopsis might have other functions, such as glucosinolate degradation, in addition to their function as leaf peroxisomes.

Table 4 Genes Specifically Expressed in Root

Gene ID	Annotation	Relative Expression Levels [a]							Peroxisomal
		Seedling	Cotyledon	Leaf	Stem	Flower	Silique	Root	Targeting Signal
At1g30990	Major latex protein (MLP)-related	0.11	0.17	0.19	0.08	0.22	0.38	7.00	PTS2
At3g13610	2-Oxoglutalate-dependent dioxygenase family	0.17	0.21	0.33	0.22	0.55	0.33	6.48	PTS1
At3g08860	Putative aminotransferase	0.20	0.42	0.48	0.37	ND[b]	ND	5.46	PTS1
At2g01880	Putative purple acid phosphatase	0.30	0.25	0.28	0.33	ND	ND	4.82	PTS1
At1g31710	Copper amine oxidase, putative	0.11	0.95	0.26	0.34	0.17	0.16	4.56	PTS2
At1g65840	Polyamine oxidase, putative	0.64	1.59	0.59	0.47	1.22	0.82	3.46	PTS1
At5g19260	Expressed protein	0.62	0.81	0.55	1.69	0.91	0.56	3.37	PTS2
At2g38760	Putative annexin	ND	ND	ND	ND	ND	ND	3.21	PTS1

[a] Ratio of signal intensity of RNA from each organ to that of RNA mixture from seven organs as a control.

[b] Not detected.

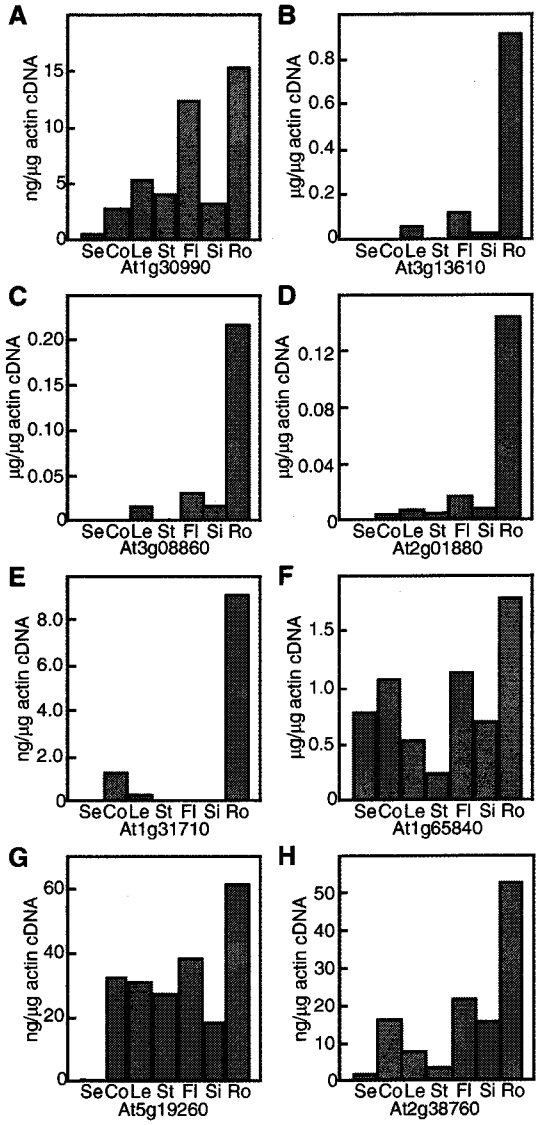

Fig. 6 Quantitative RT-PCR for transcript genes that are highly expressed in the roots. The graph shows the cDNA content of each gene whose expression is much higher in the roots than in the other organs using a microarray analysis (Table 4). The x-axis shows the quantification of transcripts by gene-specific primers relative to actin in different organs examined: (Se) seedling, (Co) cotyledon, (Le) leaf, (St) stem, (Fl) flower, (Si) silique and (Ro) root.

Table 5 Genes Specifically Expressed in Green Cotyledons

Gene ID	Annotation	Relative Expression Levels [a]							Peroxisomal
		Seedling	Cotyledon	Leaf	Stem	Flower	Silique	Root	Targeting Signal
At2g43910	Putative methyl chloride transferase	0.08	4.80	1.38	0.48	0.77	0.42	1.91	PTS2
At2g43920	Thiol methyltransferase	0.10	3.39	1.47	0.66	0.78	0.43	1.74	PTS2
At4g16410	Expressed protein	0.89	3.32	1.73	1.33	1.06	1.10	0.14	PTS2
At2g32640	Expressed protein	0.41	3.22	ND [b]	0.80	0.48	ND	0.86	PTS1
At3g04460	PEX12-like protein	0.62	3.10	0.86	1.64	0.86	1.34	0.27	- [c]

[a] Ratio of signal intensity of RNA from each organ to that of RNA mixture from seven organs as a control.

[b] Not detected.

[c] A gene whose product does not have obvious peroxisomal targeting signals.

Relationship of peroxisomes in each organ with peroxisomal gene expression profiles

To reveal the relationship of different peroxisomes in each organ, I attempted a hierarchical clustering analysis of 125 peroxisomal genes, which were the same as those used for the aforementioned K-mean clustering analysis, using GeneSpring software (Fig. 7). Consistent with the K-mean clustering analysis, peroxisomes of dark-grown seedlings, roots and cotyledons are clearly different from those of other organs. The peroxisomes of green cotyledons were characterized as genes especially expressed in green cotyledons in addition to the genes of leaf peroxisomes (Tables 3, 5). In terms of the genes encoded in leaf peroxisomal enzymes, they were especially expressed in green cotyledons, leaves and stems, but were not so much expressed in flowers and siliques (Table 3).

Five of ten genes that showed highest expression in stems are common to those in leaves (data not shown). The result suggests that leaves and stems possess functionally similar peroxisomes. In the flowers, there were three genes showed specific expression, which were genes of an AMP-binding enzyme (At1g66120) that was suggested to be identical to acyl-CoA synthetase, a cytochrom P450 like protein (At5g04660) and a putative β-alanine-pyruvate aminotransferase (At2g38400; AGT3) (data not shown) (Liepman and Olsen, 2003). The function of these genes is unknown in peroxisomes of flowers. In addition of the flower specific acyl-CoA synthetase, a fatty acid multifunctional protein (AIM1) and a 3-ketoacyl-CoA thiolase (KAT5) showed tendency of higher expression in flowers than other organs, although they existed constitutively (Table 1). There was a report that the *aim1* mutation is responsible for defective in flower development (Richmond and Bleecker, 1999), presumptively that there was a β-oxidation for a specific substrate in flowers. In green siliques, there was only one gene showed specific expression, which encode a proline rich APG like protein (At4g28780).

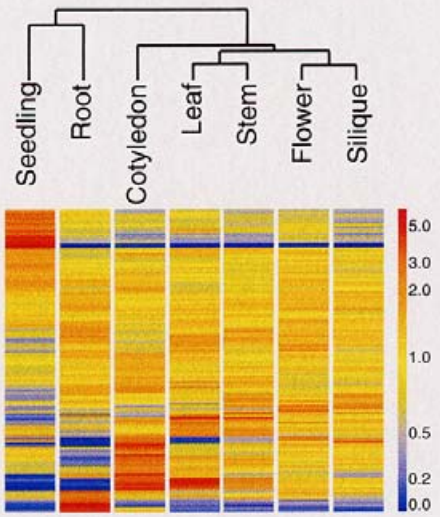

Fig. 7 Hierarchical clustering analysis of the expression profiles in the seven organs. The median of the signal ratio calculated from four repeated experiments was used for the clustering analysis using a Pearson correlation around zero. Each column represents the gene expression profile in the aforementioned organs. The signal ratio of each gene is color coded according to the scale shown to the right and organs are clustered according to the similarities of gene expression profiles. Data were analyzed using GeneSpring software.

Conclusions

By comprehensive survey of peroxisomal genes in the entire Arabidopsis genome, I found 286 candidates of peroxisomal genes. They included all genes for peroxisomal enzymes reported up to date, and most of them encoded functionally unknown proteins in peroxisomes. Overall, analyses of gene expression profiles revealed that peroxisomal differentiation is caused by the expression of specific genes that are induced in specific organs in addition with constitutive expressed genes. The clustering analyses suggested that basic functions of peroxisomes were β-oxidation, H_2O_2 degradation, BCAA catabolism and some unknown functions, and that peroxisomes in seedlings, cotyledons, leaves and roots were differentiated by organ specific expressed genes (Fig. 8). It proposed more detailed differentiation of plant peroxisomes than previous studies. Our study also showed that most genes for β-oxidation enzymes are multiple, and one set of genes is constantly expressed in all organs as constitutive peroxisomal genes, while another set is only expressed in seedlings as specified peroxisomal genes to provide differences in peroxisomes (Fig. 4). These data suggested that the functional state of plant peroxisomes is determined at the transcription level. It is well-known that reversible transformation of peroxisomes between glyoxysomes and leaf peroxisomes occurs in greening and senescence of fatty seedlings (Hayashi et al. 2000). Further analysis of the peroxisomal transition by using the peroxisomal gene array provides us the understanding of the reversibility of peroxisomes at molecular level.

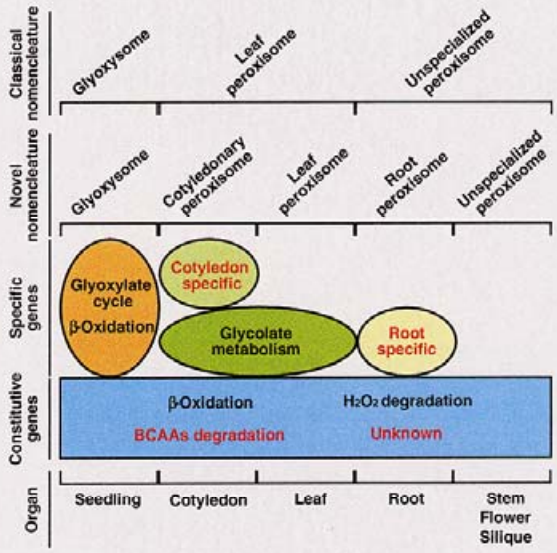

Fig. 8 Organ specific differentiation of peroxisomes in higher plants. Red letters; newly identified group of peroxisomal genes in this study.

Chapter 2

Gene Expression Profiles during Functional Transition of Peroxisomes

2.1. Introduction

During germination, the seedling must adapt its metabolic and developmental programmes to the prevailing environmental conditions to achieve photoautrophism. The light irradiation is a pivotal event in seedlings, and it is closely linked to peroxisomal functions and the functional transition. It has been suggested that the functional transformation of plant peroxisomes is controlled by gene expression, protein translocation and protein degradation, although the detailed mechanisms underlying these processes still need to be clarified (Nishimura et al., 1996). To investigate the regulation of peroxisomal functions, I analyzed gene expression profiles of germinating seedlings during irradiation following growth for 4 days in dark condition. The results showed a lot of peroxisomal genes changed the expression pattern during functional transition of glyoxysomes to leaf peroxisomes.

2.2. Materials and methods

Plant materials and growth conditions

Arabidopsis thaliana ecotype Landsberg *erecta* was used as the wild-type plant. All seeds were surface sterilized in 2% NaClO and 0.02% Triton X-100 and germinated on growth media containing 2.3 mg ml^{-1} MS salt (Wako, Osaka, Japan) 1% (w/v) sucrose, 100 μg ml^{-1} myo-inositol, 1 μg ml^{-1} thiamine-HCl, 0.5 μg ml^{-1} pyridoxine, 0.5 μg ml^{-1} nicotinic acid, 0.5 mg ml^{-1} MES-KOH (pH 5.7) and 0.8% agar. Seedlings were grown for one day in dark conditions, and after several lengths in light conditions (50 $\mu E^{-2}s^{-1}$ white light) following growth for four days under dark conditions.

RNA isolation and probe labeling

Total RNA was extracted from each tissue using the Concert^{TM} Plant RNA Reagent (Invitrogen Corp., Carlsbad, CA, USA) according to protocols provided by the manufacturer. The total RNA was further purified by precipitation with LiCl, and its quality was checked with RNA 6000 Nano LabChip kit using Agilnt 2100 Bioanalyzer (Agilent Technologies, CA, USA). For synthesis of labeled probe, Agilent Low RNA

Input Fluorescent Linear Amplification Kit (Agilent Technologies, CA, USA) was used. The total RNA (2.5 μg) was used for reverse transcription using T7 promoter primer. The cDNA was used to synthesize fluorescent cRNA using T7 RNA polymerase by *in vitro* transcription incorporating cyanine 3- or cyanine 5-CTP (Perkin-Elmer/NEN, MA, USA). For purifying labeled cRNA, I used Qiagen RNeasy mini kit (QIAGEN K.K., Tokyo, Japan) following its protocol with modifications by Agilent Technologies (Agilent Low RNA Input Fluorescent Linear Amplification Kit Manual, Agilent Technologies, CA, USA). The quality of purified labeled cRNA was checked with RNA 6000 Nano LabChip kit using Agilnt 2100 Bioanalyzer (Agilent Technologies, CA, USA), and cRNA concentration was determined by NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., DE, USA).

Microarray hybridization and data analysis

For hybridization, cyanine 3- and cyanine 5-CTP labeled cRNA (1μg each) were used with control target (*In situ* Hybridization Kit Plus, Agilent Technologies, CA, USA). The cyanine 3- and cyanine 5-CTP labeled cRNA and the control target were incubated at 60°C for 30 min with fragmentation buffer, and then hybridization buffer was added (*In situ* Hybridization Kit Plus, Agilent Technologies, CA, USA). Each experiment was repeated with reverse labeling. Arabidopsis 2 Oligo Microarrays were used for this microarray analyses including synthesized oligo-DNA fragments based on sequences of 21500 Arabidopsis genes (Agilent Technologies, CA, USA). Hybridizations were performed for 17 hr at 60°C in DNA Oven H1-80R (KURABO, Osaka, Japan) with Hybridization Chamber Assy, 22K Features (Agilent Technologies, CA, USA). The chambers were opened in a glass dish filled with 6x SSC with 0.005% TritonX-102, and then slides were washed in the same buffer at room temprature for 10 min with stirring. Slides were then rinsed in 0.1x SSC with 0.005% TritonX-102 at 4°C for 5 min with stirring and dried by Nitrogen gas using Wafergard GN Gas Filter Gun (Mykrolis, Tokyo, Japan). The fluorescent signatures were captured using an Agilent G2565BA Microarray Scanner System (Agilent Technologies, CA, USA) and analyzed using Agilent G2567AA Feature Extraction software (Agilent Technologies, CA, USA).

Some quality control procedures were conducted before data points from the replicates were averaged. First, all spots that the pixel noise of feature or background exceeds a threshold established for a uniform feature or a uniform background were

removed from the final data analysis as a non-uniformity outlier. Second, two kinds of test were conducted to determine if the mean signal of a spot is greater than the background. One test determines if the difference is significant by a 2-sided t-test against p-value 0.01 (IsPosAndSignif test), and the other test determines if the net signal following the subtraction of the background from the raw mean signal is greater than 2.6x background standard deviation. All spot that the signal is determined less than the background or the difference is not significant are eliminated from the final data analysis. A LOWESS dye normalization method is used for the normalization of the red and green channels. If the net signal following the subtraction of the background from the raw mean signal fails the IsPosAndSignif test or is less than its background standard deviation, a surrogate value is calculated as one standard deviation of the background multiplied by the dye normalization factor and used as the "lowest limit of detection" to replace the dye normalized signal. Log ratio is calculated between the red and green channels and the log ratios from repetitious experiments are averaged only when the error of repetitious data is in 2-hold threshold. The statistical significance on the each log ratio was calculated, as the p-value is less than 0.01, which means you can say with a 99% confidence level that the gene is differentially expressed.

Quantitative RT-PCR analysis

Total RNA from Arabidopsis seedlings, which transferred to light conditions were grown after following growth for four days under dark conditions, was extracted using the same method as for the microarray (see above). The first strands from 5 μg total RNA in the 20 μL reaction mixture were synthesized using the SUPERSCRIPT™ First-Strand Synthesis system for RT-PCR (Invitorgen Corp., Carlsbad, CA, USA) according to the manufacturer's protocol using oligo(dT) primers. The PCR was performed using the SmartCycler Instrument (Cepheid, CA, USA) with the SYBR Green I (BioWhittaker Molecular Applications, ME, USA) and the Takara Ex Taq™ R-PCR Version kit for PCR (Takara, Shiga, Japan) according to the manufacturer's protocol. Each reaction was performed on 2 μL of a 10 times dilution of the first cDNA strands and synthesized as described above in a total reaction of 25 μL. Specific primer sets were designed for each gene (see supplemental data). The reactions were incubated at 95°C for 30 sec to activate the hot-start recombinant *Taq* DNA polymerase, followed by 40 cycles of 3 sec at 95°C and 20 sec at 68°C to measure the fluorescence signal. The

specificity of the PCR amplification procedures was checked with a heat dissociation protocol (from 65°C to 95°C) after the final cycle of the PCR.

2.3. Results and discussion

Microarray analysis

First, I investigated developmental changes in the levels of transcripts of isocitrate lyase and hydroxypyruvate reductase by quantitative RT-PCR, which is an enzyme for the glyoxylate cycle and for glycolate metabolism respectively (Fig. 9). The level of transcripts of isocitrate lyase was abundant immediately after start incubation following cold treatment. It was gradually decreased and negligible after illumination. On the other hand, the level of transcripts of hydroxypyruvate reductase was nearly undetectable in dark condition, and it was induced by light. The results were good agreement with the previous reports, showing that the level of transcripts of hydroxypyruvate reductase reached maximum level within 1 day on light condition.

To investigate gene expression profiles during the functional transition of peroxisomes, I analyzed gene expression profiles of cotyledons grown after 2hr, 6hr, 12hr or 24hr in light conditions following growth for 4 days under dark conditions (4D2hrL, 4D6hrL, 4D12hrL and 4D24hrL) based on that of cotyledons grown for 4 days in dark conditions (4D) using Agilent Arabidopsis 2 Oligomicroarrays. In order to reveal gene expression profiles shortly after onset of germination, RNA from seedlings grown for 1 day under dark condition (1D) was also compared with that of cotyledons grown in dark conditions for 4 days.

Down-regulated genes during illumination

It is well known that genes of glyoxysomal proteins were reduced in light. I searched genes that regulated in concert with glyoxysomal genes. I performed to collect genes showed similar expression pattern with that of two glyoxysomal enzymes, namely 3-ketoacyl-CoA thiolase (At2g33150) and malate synthase (At5g03860), during illumination by GeneSpring software. Both of them were repressed during illumination in this microarray data. Hierarchical cluster analysis is popular to find a set of genes showing similar expression pattern. In the hierarchical tree calculated with all positive data from 21500 genes (Fig. 10A), the 3-ketoacyl-CoA thiolase and malate synthase were divided at root of the tree. It conflict with the data. So I used a statistical method, namely standard correlation using only data from genes that showed positive data in

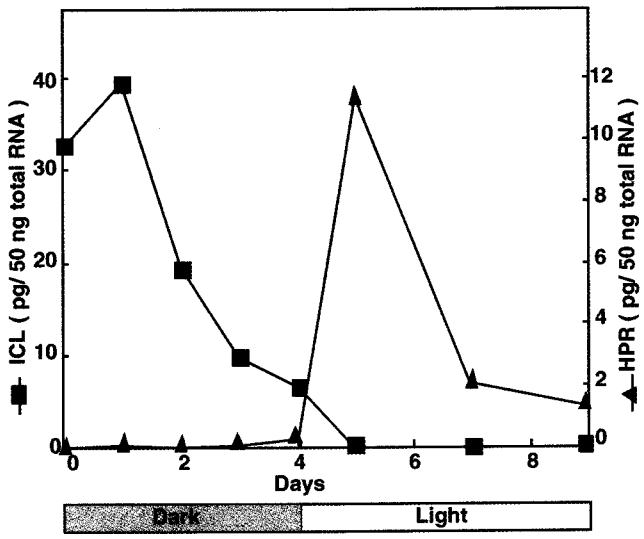

Fig. 9 Developmental changes in the level of mRNAs for isocitrate lyase (ICL) and hydroxypyruvate reductase (HPR). The quantitative RT-PCR was performed to determine the quantity of transcripts using gene-specific primers. Wild-type plants were grown at dark condition until 4 days after onset of germination and transferred to continuous illumination.

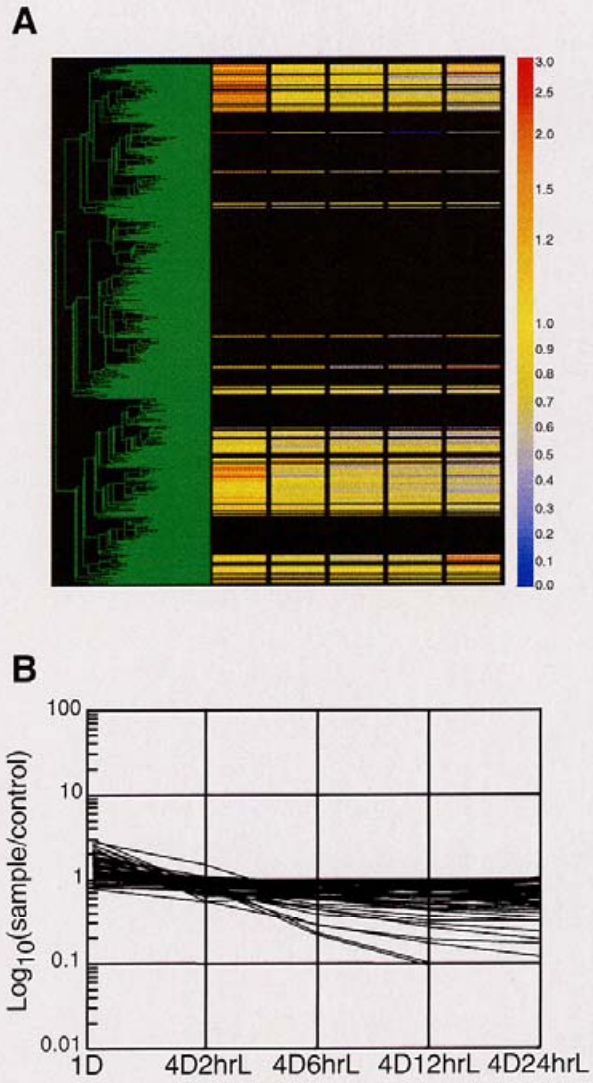

Fig. 10 Genes concomitantly expressed with those of 3-ketoacyl-CoA thiolase and malate synthase. (A): Hierarchical cluster analysis of the expression patterns of *Arabidopsis* 21500 genes (arrayed along the vertical axis) at different time during development after illumination (horizontal axis). *Arabidopsis* 21500 genes are grouped according to similarities in expression profiles at left tree. Log_{10} signal ratios of 2593 genes, which showed similar expression pattern with 3-ketoacyl-CoA thiolase and malate synthase, are color coded according to the scale at right, (B): Expression profiles of 50 peroxisomal genes that included in the group showed (A). The y-axis is Log_{10} signal ratio of each time point based on the signal intensity of RNA from seedlings grown for 4 days in dark condition.

more than half of the five time point data sets (see Materials and methods). It was calculated accuracy with patterns similarity. In the threshold value was 0.8, I got 2736 genes at first. In the 2736 genes, it was included that genes do not have positive data in the time point 1D based on 4D. It could not be confirmed that the pattern is light dependent or not in such case. So I excluded genes that the signal is not positive in 1D, and total 2593 genes were collected. It included all genes for reported enzymes of glyoxysomes (Fig. 10A, Table 6). Genes in this group reduced the amount of mRNAs during illumination. The 2593 genes included 50 genes of which were mentioned as genes encoding peroxisomal protein in the chapter 1 (Fig. 10B, Table 6). The At2g39970, At5g11520 and At5g42890 gene, which were suggested to function in glyoxysomes in Chapter 1, showed synchronized expression pattern with those of established glyoxysomal genes (Table 6). Although functions of the other peroxisomal genes in this group were unknown, it is possible that they use a common mechanism of gene regulation and to function in seedlings.

Up-regulated genes during illumination

In the same manner, I collected genes which showed similar expression pattern with genes encoding the enzymes for glycolate pathway, namely glycolate oxidase (At3g14420) and hydroxypyruvate reductase (At1g68010). With a value of standard correlation, 0.8, I got 3139 genes at first. I excluded genes that the signal is not positive in 1D, and then total 2914 genes were collected (Fig. 11A). Genes in this group were induced during illumination. The 2914 genes included 48 genes of which were mentioned as encoding peroxisomal protein in the chapter 1. It included almost of all genes for reported enzymes of glycolate metabolism (Fig. 11B, Table 7).

Only an aminotransferase gene (At1g70580) for glycolate metabolism do not include in this group. It is because that the ratios of the gene expression in 1D based on 4D and in 4D2hrL based on 4D were almost same. Although, the expression pattern was light induced one as same as that of the other genes for glycolate metabolism. The results is not conflicted one between the result of chapter 1 that the gene show high expression in greening cotyledons (Table 3). This gene was suggested to be regulated the expression by a different mechanism from other leaf peroxisomal genes. Along with the expression pattern of these genes for glycolate metabolism, At2g32640, At2g43910 and At4g16410 also showed up-regulated expression pattern by light (Table 7), which

Table 6 Peroxisomal genes concomitantly expressed with those of 3-ketoacyl-CoA thiolase and malate synthase

Gene ID	Annotation	Relative Expression Levels [a]				
		1D	4D2hrL	4D6hrL	4D12hrL	4D24hrL
β-Oxidation						
At1g04710	KAT1	0.23	-0.03	-0.19	-0.20	-0.19
At1g06290	ACX3	0.27	0.00	-0.16	-0.20	-0.28
At1g06310	Acyl-CoA oxidase-like	0.29	0.04	-0.16	-0.21	-0.23
At2g33150	PED1/ KAT2	0.28	-0.10	-0.41	-0.53	-0.62
At3g06860	AtMFP2	0.35	-0.10	-0.54	-0.71	-0.77
At5g27600	AtLACS7	0.33	-0.08	-0.35	-0.49	-0.50
At5g65110	ACX2	0.26	-0.12	-0.22	-0.37	-0.47
At4g29010	AIM1	0.06	0.04	-0.07	-0.03	-0.15
At5g16370	Acyl CoA synthetase	0.16	-0.12	-0.20	-0.20	-0.14
At3g15290	3-Hydroxybutyryl-CoA dehydrogenase putative	-0.08	-0.10	-0.15	-0.19	-0.23
At5g43280	Enoyl CoA hydratase-like protein	0.04	-0.05	0.00	-0.06	-0.18
At5g65940	CHY1	0.08	0.01	-0.13	-0.02	-0.12
At2g30650	3-Hydroxyisobutyryl-coenzyme A hydrolase	0.10	0.04	-0.05	-0.05	-0.10
Glyoxylate Cycle						
At2g05710	Cytoplasmic aconitate hydratase	0.45	0.18	-0.24	-0.30	-0.37
At2g22780	Putative glyoxysomal malate dehydrogenase precursor	0.42	-0.17	-0.32	-0.44	-0.51
At3g21720	Putative isocitrate lyase	-0.06	0.00	-0.63	-0.99	- [b]
At3g58750	Citrate synthase -like protein	0.05	-0.02	-0.04	-0.05	-0.21
At5g03860	Malate synthase -like protein	0.41	-0.07	-0.65	-1.02	-
At2g42790	Putative citrate synthase	0.39	-0.24	-0.54	-0.76	-0.92
At4g35830	Cytoplasmatic aconitate hydratase	0.01	0.04	-0.13	-0.06	-0.08
Other Glyoxysomal Genes						
At2g39970	PMP38	0.07	-0.01	-0.20	-0.34	-0.28
At5g11520	Asp3	0.49	-0.09	-0.29	-0.35	-0.40
At5g42890	Expressed protein	0.08	-0.15	-0.35	-0.57	-0.71

[a] Log_{10} ratio of signal intensity of RNA from each seedlings illuminated for several time length to that of RNA from seedlings grown for 4 day in dark condition.

[b] Not positive.

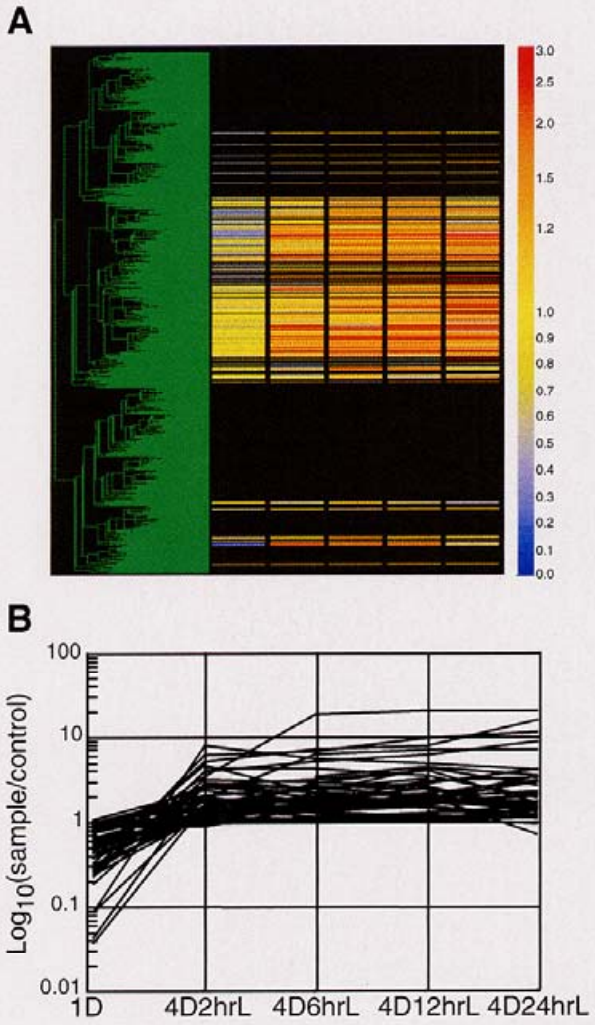

Fig. 11 Genes concomitantly expressed with those of hydroxypyruvate reductase and glycolate oxidase. (A): Hierarchical cluster analysis of the expression patterns of *Arabidopsis* 21500 genes (arrayed along the vertical axis) at different time during development after illumination (horizontal axis). *Arabidopsis* 21500 genes are grouped according to similarities in expression profiles at left tree. Log_{10} signal ratios of 2914 genes, which showed similar expression pattern with hydroxypyruvate reductase and glycolate oxidase, are color coded according to the scale at right, (B): Expression profiles of 48 peroxisomal genes that included in the group showed (A). The y-axis is Log_{10} signal ratio of each time point based on the signal intensity of RNA from seedlings grown for 4 days in dark condition.

Table 7 Peroxisomal genes concomitantly expressed with those of hydroxypyruvate reductase and glycolate oxidase

Gene ID	Annotation	Relative Expression Levels [a]				
		1D	4D2hrL	4D6hrL	4D12hrL	4D24hrL
Glycolate Metabolism						
AT1G23310	AtGGT1	-0.48	0.72	0.87	0.89	1.21
AT1G68010	Hydroxypyruvate reductase	-0.65	0.60	0.72	0.80	0.95
AT2G13360	AGT1	-0.57	0.79	0.84	0.99	1.06
AT3G14415	Glycolate oxidase	0.00	0.32	0.40	0.52	- [b]
AT3G14420	Glycolate oxidase, putative	-0.55	0.44	0.51	0.61	-
AT5G09660	Malate dehydrogenase NAD-dependent	-1.06	0.19	0.28	0.39	-
Other Cotyledonary Genes						
AT2G32640	Expressed protein	-0.44	-	0.54	0.65	0.55
AT2G43910	Putative methyl chloride transferase	-1.06	0.91	0.76	0.69	0.62
AT4G16410	Expressed protein	-0.58	0.20	0.27	0.28	-
Protease						
AT5G42620	Major surface glycoprotein-like	-0.12	0.16	0.07	0.03	-
AT5G47040	Lon protease homolog 1 precursor	0.03	0.38	0.25	0.28	0.54

[a] Log_{10} ratio of signal intensity of RNA from each seedlings illuminated for several time length to that of RNA from seedlings grown for 4 days in dark condition.

[b] Not positive.

genes were reported as cotyledon specific genes (Table 5).

Functions of the other peroxisomal genes in this group were unknown. Though, there were two peroxisomal genes, At5g42620 and At5g47040, which products were predicted to have a domain with protease activity (in TAIR; http://www.arabidopsis.org/). In the process of functional transition, glyoxysomal enzymes were degraded, in addition to repressed expression of glyoxysomal genes. Although the function of proteases in greening cotyledons is unknown, these gene products can be candidates for proteases functioned at the process of peroxisomal functional transition.

Comparison of expression profiles of isogenes during development

As mentioned in chapter 1, some glyoxysomal enzymes were coded with two or three genes. It was suggested that the isogenes for β-oxidaiton enzymes could be divided into two groups by the expression, namely seedling type and constitutive type in chapter 1 (Table1, Table 2, Fig. 4). The results of gene expression during illumination were almost consistent with chapter 1 in terms of isognes (Table 8). The genes of seedling type were expressed acutely in 1D and reduced during illumination. On the other hand, almost of genes in constitutive type showed not so much change of transcripts level during illumination. It was also correspond to the previous results that a gene of malate dehydrogenase (At2g22780) was expressed in seedlings then reduced, and the other one (At5g09660) was induced during illumination (Fig. 4E, Table 8).

In addition of those results, the expression of ACX3 isogene (At1g06310) and ACX1 isogene (At2g35690) were expressed concomitantly with the genes of ACX3 and ACX1, respectively. It could not have been confirmed the level of transcripts of those isogenes because of their low expression in RT-PCR analysis in chapter 1 (Fig. 4A, 4B). One of the enzymes for glyoxylate cycle, citrate synthase, is also coded with three isogenes. One of the isogenes (At2g42790) showed the expression pattern of seedling type, and others showed constitutive type (At3g58740 and At3g58750). In contrast with genes for β-oxidation, the other genes of glyoxylate cycle do not have isogenes. So the isogenes of citrate synthase expressed constitutively may be functioned for other metabolic pathways. One of the citrate synthase isogenes, At3g58740, has been divided into glyoxysomal group in chapter 1 (Table 2) in conflict with this result (Table 8). One possible reason is the cross-hybridization. It could not be confirmed the gene expression

Table 8 Developmental changes in the level of mRNA for isogenes

Gene ID	Annotation	Relative Expression Levels [a]					Relative Expression Levels [b]	
		1D	4D2hrL	4D6hrL	4D12hrL	4D24hrL	seedling	cotyledon
Seedling type								
At5g65110	ACX2	0.26	-0.12	-0.22	-0.37	-0.47	3.69	0.68
At1g06290	ACX3	0.27	0.00	-0.16	-0.20	-0.28	2.80	0.72
At1g06310	ACX3 isogene	0.29	0.04	-0.16	-0.21	-0.23	2.99	0.67
At3g51840	ACX4	0.33	0.10	0.04	-0.10	-0.12	2.07	0.86
At3g06860	AtMFP2	0.35	-0.10	-0.54	-0.71	-0.77	4.69	0.38
At2g33150	PED1/ KAT2	0.28	-0.10	-0.41	-0.53	-0.62	4.01	0.52
At1g04710	KAT1	0.23	-0.03	-0.19	-0.20	-0.19	3.48	0.66
At2g42790	CS	0.39	-0.24	-0.54	-0.76	-0.92	-[c]	-
At2g22780	MDH	0.42	-0.17	-0.32	-0.44	-0.51	3.67	0.90
Constitutive type								
At4g16760	ACX1	-0.10	0.11	-0.14	-0.26	-	1.24	0.98
At2g35690	ACX1 isogene	-0.09	0.03	0.01	-0.02	0.03	1.03	0.90
At4g29010	AIM1	0.06	0.04	-0.07	-0.03	-0.15	0.75	1.12
At5g48880	KAT5	-1.37	0.47	0.41	0.31	-0.15	0.39	1.04
At3g58740	CS	-	0.01	0.03	0.10	-	2.05	0.69
At3g58750	CS	0.05	-0.02	-0.04	-0.05	-0.21	1.71	0.70
Cotyledon type								
At5g09660	MDH	-1.06	0.19	0.28	0.39	-	0.21	3.65

[a] Log_{10} ratio of signal intensity of RNA from each seedlings illuminated for several time length to that of RNA from seedlings grown for 4 day in dark condition.

[b] Ratio of signal intensity of RNA from each organ to that of RNA mixture from seven organs as a control. The results in chapter 1.

[c] Not positive.

profile in quantitative RT-PCR analysis because a set of specific primer for the gene could not be constructed. In terms of the other two citrate synthase gene, they were confirmed the expression profiles in various organs by quantitative RT-PCR analysis (data not shown). The data were accord with this time series experiment, which At2g42790 was expressed in seedling specifically, and At3g58750 was expressed constitutively.

Interestingly, the KAT5 encoding a 3-ketoacyl-CoA thiolase showed transitory expression (Table 8). It have been suggested that the pathway of β-oxidation play some role in production of a lipid signaling molecule, like jasmonic acid (Richmond and Bleeker, 1999). The gene was possible to play some roles in the functional transition of peroxisomes.

Genes functioned during the functional transition in peroxisomes

To investigate the genes functioned during functional transition of peroxisomes, I surveyed genes showed transitory expression, like KAT5. In the same manner with glyoxysomal-like genes mentioned above, I collected genes that showed similar expression pattern with KAT5 gene (At5g48880), though the gene function is still unknown. With a value of standard correlation, 0.8, I got 30 peroxisomal genes. I picked up only genes that the all signals in time series were positive and the data show an arched shape. Total 9 genes were collected including KAT5 itself (Table 9). Genes in this group were induced during illumination, and reduced at the variety of rate. Functions of all 9 genes are not known up to date. The 3 of 9 genes (At1g31710, At1g65840 and At2g38760) were divided into root specific group in chapter 1. Those genes may function at developmental cells. The 2 of the 9 genes (At1g48320 and At5g48880) were divided into constitutive group including KAT1, and one (At2g43910) was cotyledonary. The others were not grouped in chapter 1. These nine peroxisomal genes were candidates that play some roles of the functional transition in peroxisomes.

Table 9 Genes concomitantly expressed with those of *KAT5*

Gene ID	Annotation	Relative Expression Levels [a]				
		1D	4D2hrL	4D6hrL	4D12hrL	4D24hrL
At1G31710	Copper amine oxidase, putative	-0.83	0.08	0.18	0.11	-0.05
At1G48320	Thioesterase family	-0.52	0.25	0.24	0.16	0.04
At1G65840	Polyamine oxidase, putative	-0.60	0.51	0.48	0.42	0.14
At2G38760	Putative annexin	-0.88	0.21	0.08	-0.05	-0.21
At2G43910	Putative methyl chloride transferase	-1.06	0.91	0.76	0.69	0.62
At3G04290	Putative GDSL-motif lipase/acylhydrolase	-1.43	0.21	0.43	0.39	0.14
At3G51740	Leucine-rich repeat transmembrane protein kinase, putative	-0.42	0.13	0.31	0.34	0.11
At5G37670	Low-molecular-weight heat shock protein - like	-0.17	0.13	0.12	-0.01	-0.02
At5G48880	KAT5	-1.37	0.47	0.41	0.31	-0.15

[a] Log_{10} ratio of signal intensity of RNA from each seedlings illuminated for several time length to that of RNA from seedlings grown for 4 days in dark condition.

2.4. Conclusions

To reveal the gene expression profiles during functional transition of peroxisomes, I performed microarray analysis of time series during irradiation. In this experiment, I collected genes showed similar expression pattern with established glyoxysomal and leaf peroxisomal genes during illumination, respectively. At least, 48 peroxisomal genes were up-regulated and another 50 peroxisomal genes were down-regulated during the functional transition of glyoxysomes to leaf peroxisomes. Of these, two proteins possessing a domain of protease activity were included in light inducible genes. These are candidates of proteases functioned in the functional transition of peroxisomes, such as degradation of glyoxysomal proteins. Although, almost of the genes are not known their function, they are considered to share some regulation mechanisms of gene expression.

Between isogenes, the differential expression patterns were almost consistent with this experiment. Among them, one of 3-ketoacyl-CoA gene, *KAT5*, were showed transitory expression pattern. I collected genes showed such transitory pattern based on the *KAT5* and got nine peroxisomal genes. Those genes were considered to play some role during the functional transition of peroxisomes.

In the result of this study, I got these groups of genes, up-regulated one, down-regulated one and transitory expressed one. It will give a basic knowledge to reveal mechanisms of functional transition in peroxisomes.

General Discussion

To obtain comprehensive vision of an organelle is ideal approach to recognize the organelle. Arabidopsis is one of the plants that are suitable for exhaustive analysis. Because it has been established the information of the complete genome sequences. I performed comprehensive survey of peroxisomal genes in the entire Arabidopsis genome, and found 286 candidates of peroxisomal genes, most of which encoded functionally unknown proteins in peroxisomes.

. The peroxisomal targeting signals of mammals, yeasts and trypanosomes have also been analyzed, and the divergence in permissible tripeptide sequences among these organisms has been discussed (Purdue and Lazarow, 1994). Comparison of mammalian consensus sequences with that for plants revealed that there are some features specifically for plant peroxisomal targeting signals (Hayashi et al., 1997b). In this reason, I performed the prediction of peroxisomal proteins based on the amino acid sequences reported in Arabidopsis (Hayashi et al., 1997a) I also searched PTS2-containing proteins by a conserved region in amino terminal sequences (Kato et al., 2000) up to the 50th amino acid residues from the N-terminal. These search are considered of values because they included all genes for peroxisomal enzymes containing PTS reported up to date. Further analysis is needed to confirm the localization *in vivo* more directly. On the other hand, the possibility is left that there are some peroxisomal genes excluding in this search. In the proteomic analyses of leaf peroxisomes and glyoxysomes, some PTS1-like and PTS2-like proteins were identified in isolated peroxisomes (Fukao et al., 2002; Fukao et al., 2003). Based on the results, it may possible to be some other candidates of signal sequence, like PTS1 or PTS2, or some other transport mechanisms, like catalases (Kamigaki, et al., 2003). Furthermore, the mechanisms of transport of peroxisomal membrane proteins are less known. To join in with proteomic analyses will be important tool to identify peroxisomal proteins comprehensibly.

It has long been recognized that peroxisomes in higher plants are differentiated in function depending on the cell type. In previous study, only limited number of genes could be analyzed their function and expression at one time. This study, which comprehensively survey of peroxisomal genes and microarray analyses focused on those genes, revealed more diversity of the plant peroxisomes. It was accomplished because these comprehensive analyses give some gene sets based on their expression profiles without intension.

The regulation of peroxisomal gene expression was well studied in mammals. In mammals, peroxisomal β-oxidation genes are induced by peroxisome proliferators (PPs), which are a general class of compounds that increase both the number and size of peroxisomes in treated mammalian cells (Corton et al., 2000). PPs act through a family of nuclear receptors/transcription factors called PPARs for peroxisome proliferator-activated receptors. Binding of PPs to PPARs targets them to promoter elements in β-oxidation genes known as peroxisome proliferator response elements (PPREs). In Arabidopsis genome, I failed to search the PPARs orthologues based on amino acid sequences of human PPARα, β and γ by PSI-BLAST.

The regulation mechanisms of peroxisomal gene expression have not yet been established in plants. There are only some reports on *cis*-acting elements of some plant peroxisomal genes. Distinct *cis*-acting elements have been discovered within the promoters of the malate synthase (Sarah et al., 1996) and isocitrate lyase genes (De Bellis et al., 1997) that mediate the expression responses to germination and sugar signals. The promoter of the parsley homologue of ACX2 has been isolated and shown to possess several *cis*-acting elements including a sugar-response element (Logrmann et al., 2000). It is complicated with variety of regulatory signals. The microarray analyses may represent a step on the way to reveal such regulation mechanisms of peroxisomal gene. In the chapter 2, for example, I got nine genes showed transitory expression during illumination. The three of nine genes belong to another group showed root specific expression in chapter 1. Based on these results, I can narrow the search to the common ground of two conditions. It is also thought that the gene expression regulated by the two conditions independently. For the way to exclude that possibility, accumulation of microarray data in various conditions is thought to be effective to concentrate massive data sets.

Microarray analysis is landmark to provide an overall picture of the genome expressions. In quiet recently, the technique have made considerable progress in data stability, especially with respect to microarrays consisted of synthesized oligo DNA. This study proposed that there is more functional diversity of plant peroxisomes, although the function is still unknown. From the microarray analyses, I got some candidates to reveal unidentified peroxisomal function. Further physiological analyses will give us a deeper understanding of peroxisomal function and the mechanisms of functional transition.

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Sapplemental Data 1 List of Peroxisomal Genes and the Construction of Peroxisome Specific Microarray

Meta Row*	Meta Column*	Row*	Column*	Reporter ID	Reporter Usage*	Control Type	Cluster#	Gene ID	Sequence ID	Annotation	Signal	Reference#
1	1	1	2	1	Experimental	-	1	ch_e_29	at5g27600	long-chain-fatty-acid-CoA ligase-like protein (acyl CoA synthetase)	PTS1,2	1,2
1	2	1	2	2	Experimental	-	1	f2o10	at3g05970	long-chain-fatty-acid-CoA ligase putative (acyl CoA synthetase)	PTS2	1,2
1	2	1	3	4	Experimental	-	2	mqk4	at5g16370	AMP-binding protein, putative (acyl CoA synthetase)	PTS1	
1	1	1	4	5	Experimental	-	2	k14a17	at3g16910	AMP-binding protein putative (acyl CoA synthetase)	PTS1	
3	1	3	9	153	Experimental	-	3	f2d10	at1g20560	AMP-dependent synthetase and ligase family	PTS1	
1	1	4	10	167	Experimental	-	3	f15e12	at1g66120	AMP-dependent synthetase and ligase family	PTS1	
1	1	5	10	283	Experimental	-	3	f12p19.5	at1g65880	AMP-dependent synthetase and ligase family	PTS1	
-	-	-	-	284	-	-	3	f12p19.6	at1g65890	AMP-binding protein, putative	PTS1	
3	1	1	2	11	Experimental	-	4	fca_all	at4g16760	acyl-CoA oxidase (ACX1)	PTS1	32
3	2	1	2	12	Experimental	-	4	t20f21	at2g35690	acyl-CoA oxidase putative	PTS1	32
1	1	1	6	9	Experimental	-	5	t2d23	at1g06310	acyl-CoA oxidase like	PTS2	3,4
3	2	5	10	296	Experimental	-	5	f9p14	at1g06290+at1g0630	acyl-CoA oxidase (ACX3)	PTS2	3,4
3	1	1	3	13	Experimental	-	6	dt_d_28	at4g29010	fatty acid multifunctional protein (AIM1)	PTS1	5,6
3	2	1	3	14	Experimental	-	6	f17a9	at3g06860	fatty acid multifunctional protein (AtMFP2)	PTS1	5,6
3	2	1	4	16	Experimental	-	7	t11j7	at2g30650	3-hydroxyisobutyryl-coenzyme A hydrolase	PTS1	7
3	1	1	5	17	Experimental	-	7	t11j7	at2g30660	3-hydroxyisobutyryl-coenzyme A hydrolase	PTS1	7
3	2	1	5	18	Experimental	-	7	k14b20	at5g65940	3-hydroxyisobutyryl-coenzyme A hydrolase (CHY1)	PTS1	7
1	1	2	1	21	Experimental	-	8	k24g6	at5g48880	3-ketoacyl-CoA thiolase (KAT5)	PTS2	8,9
1	2	2	1	22	Experimental	-	8	f25i18	at2g33150	3-ketoacyl-CoA thiolase (PED1/ KAT2)	PTS2	8,9
1	2	2	2	24	Experimental	-	8	t1g11	at1g04710	acetyl-CoA acyltransferase putative (KAT1)	PTS2	8,9
1	1	2	4	27	Experimental	-	9	ve_c_26	at3g58740	citrate synthase -like protein	PTS2	10
1	2	2	4	28	Experimental	-	9	ve_c_26	at3g58750	citrate synthase -like protein	PTS2	10
1	1	2	5	29	Experimental	-	9	f7d19	at2g42790	citrate synthase putative	PTS2	10
1	2	2	5	30	Experimental	-	10	dt_e_22	at5g09660	malate dehydrogenase microbody NAD-dependent	PTS2	11
1	1	2	6	31	Experimental	-	10	t30l20	at2g22780	malate dehydrogenase precursor putative glyoxysomal	PTS2	11
3	1	2	2	35	Experimental	-	11	T3P4	at2g05710	cytoplamic aconitate hydratase	-	12
3	2	2	2	36	Experimental	-	11	ks_d_20	at4g26970	putative aconitase	-	12
-	-	-	-	310	-	-	11	en_d_23	at4g35830	aconitate hydratase	-	12
3	1	2	5	41	Experimental	-	12	f5a18	at1g70580	putative alanine aminotransferase (AtGGT2)	PTS1	13
3	2	2	5	42	Experimental	-	12	f26f24	at1g23310	putative alanine aminotransferase (AtGGT1)	PTS1	13
3	1	2	6	43	Experimental	-	13	t16o11	at3g08860	putative aminotransferase	PTS1	
3	2	2	6	44	Experimental	-	13	t19c21	at2g38400	beta-alanine-pyruvate aminotransferase putative (AGT3)	PTS1	
3	1	3	7	149	Experimental	-	13	ph_d_20	at4g39660	alanin aminotransferase (AGT2)	PTS1	
1	1	3	2	47	Experimental	-	14	ge_c_28	at3g48730	glutamate-1-semialdehyde aminotransferase	PTS1	
3	1	2	10	131	Experimental	-	14	mbk5	at5g63570	glutamate-1-semialdehyde 2,1-aminomutase 1 precursor	PTS1	
1	2	3	2	48	Experimental	-	15	by_d_20	at4g35090	catalase2	-	14
1	1	3	3	49	Experimental	-	15	f5m15	at1g20620	catalase3	-	14
1	2	3	3	50	Experimental	-	15	f5m15	at1g20630	catalase1	-	14
1	1	4	1	69	Experimental	-	16	ge_c_27	at3g47430	pex11 like	-	

3	2	2	8	128	Experimental	-	16	t2e6	at1g47750	pex11 like	-
1	1	4	3	73	Experimental	-	17	t21p5	at3g03490	pex19 like	-
1	2	4	3	74	Experimental	-	17	ku_e_29	at5g17550	pex19 like	-
3	2	4	1	82	Experimental	-	18	ge_c_21	at3g48100	response reactor 2 (ATRR2)	PTS1
3	1	4	2	83	Experimental	-	18	mqb2	at5g62920	response regulator 6 (ARR6)	PTS1
3	2	4	2	84	Experimental	-	19	mpo12	at5g40310	zinc finger -like protein	PTS2
3	1	5	4	203	Experimental	-	19	k24a2	at3g27970	exonuclease protein family	PTS2
1	2	1	8	96	Experimental	-	20	ch_d_27	at4g05160	4-coumarate-CoA ligase - like protein	PTS1
1	1	1	9	97	Experimental	-	20	pl_d_20	at4g19010	4-coumarate-CoA ligase - like	PTS1
1	2	1	9	98	Experimental	-	20	k9h21	at5g63380	4-coumarate-CoA ligase-like protein	PTS1
3	1	3	11	157	Experimental	-	20	f5m15	at1g20510	4-coumarate:CoA ligase 1 (4-coumaroyl-CoA synthase 1) (4CL1) fa	PTS1
3	2	3	11	158	Experimental	-	20	f5m15	at1g20500	4-coumarate:CoA ligase 1 (4-coumaroyl-CoA synthase 1) (4CL1) fa	PTS1
3	1	3	12	159	Experimental	-	20	f5m15	at1g20490	4-coumarate:CoA ligase 1 (4-coumaroyl-CoA synthase 1) (4CL1) fa	PTS1
-	-	-	-	160	-	-	20	f5m15	at1g20480	4-coumarate:CoA ligase 1 (4-coumaroyl-CoA synthase 1) (4CL1) fa	PTS1
1	1	1	10	99	Experimental	-	21	mah20	at5g08460	GDSL-motif lipase/acylhydrolase-like protein	PTS1
1	2	1	10	100	Experimental	-	21	t6k12	at3g04290	putative GDSL-motif lipase/acylhydrolase	PTS1
1	1	1	11	101	Experimental	-	21	t20k24	at2g19050	putative GDSL-motif lipase/hydrolase	PTS1
1	1	3	7	137	Experimental	-	21	bt_d_22	at4g28780	Proline-rich APG - like protein	PTS1
3	2	6	2	224	Experimental	-	21	pl_d_20	at4g18970	GDSL-motif lipase/hydrolase protein	PTS1
1	1	7	1	233	Experimental	-	21	ku_e_23	at5g15720	GDSL-motif lipase/hydrolase protein	PTS1
3	1	1	8	105	Experimental	-	22	mfl8	at2g43020	putative amine oxidase	PTS1
3	1	1	9	107	Experimental	-	22	f1e22	at1g65840	polyamine oxidase, putative	PTS1
1	2	6	4	216	Experimental	-	22	ve_c_24	at3g59050	similar to polyamine oxidase	PTS1
1	1	2	7	113	Experimental	-	23	f6e13	at2g43910	putative methyl chloride transferase	PTS2
3	2	8	6	276	Experimental	-	23	f6e13	at2g43920	thiol methyltransferase	PTS2
1	1	2	9	117	Experimental	-	24	f2n1	at4g01190	putative phosphatidylinositol kinase	PTS2
1	2	5	3	190	Experimental	-	24	f22l4	at1g01460	4,5 PIP kinase -related	PTS2
3	1	8	6	275	Experimental	-	24	f17j6	at1g50510	expressed protein	PTS2
1	1	3	12	147	Experimental	-	25	f11a17	at1g48320	thioesterase family	PTS1
1	2	7	5	242	Experimental	-	25	k19e20	at5g48950	thioesterase-related	PTS1
1	2	5	5	194	Experimental	-	26	f27m3	at1g31710	copper amine oxidase -related	PTS2
1	1	5	6	195	Experimental	-	26	f27m3	at1g31690	copper amine oxidase -related	PTS2
-	-	-	-	204	-	-	27	rr_c_26	at3g42490	hypothetical protein	PTS2
3	2	7	4	252	Experimental	-	27	mee13	at5g36070	hypothetical protein	PTS2
1	2	5	12	288	Experimental	-	27	t32g9	at1g35070	hypothetical protein	PTS2
3	1	6	1	221	Experimental	-	28	wt_d_32	at4g39140	expressed protein	PTS1
1	2	8	6	266	Experimental	-	28	f3k23	at2g21500	expressed protein	PTS1
1	1	7	2	235	Experimental	-	29	ch_e_53	at5g33340	hypothetical protein	PTS1
1	2	7	2	236	Experimental	-	29	ch_e_53	at5g33350	putative protein	PTS1
3	1	7	5	253	Experimental	-	30	k6o8	at5g53840	F-box protein family	PTS2
1	1	5	9	281	Experimental	-	30	mdj22	at5g22660	F-box protein family	PTS2
1	2	5	11	286	Experimental	-	30	t13d8	at1g60400	F-box protein family	PTS2

1	1	5	12	287	Experimental	-	30	t13d8	at1g60410	F-box protein family	PTS2		
1	1	8	3	259	Experimental	-	31	f1k23	at1g28710	expressed protein	PTS1		
1	2	8	3	260	Experimental	-	31	f1k23	at1g28700	hypothetical protein common family	PTS1		
1	2	5	9	282	Experimental	-	31	mhk7	at5g40900	hypothetical protein common family	PTS2		
-	-	-	-	26	-	-	32	fca_all	at4g14430	carnitine racemase like protein	PTS1		
1	2	4	9	166	Experimental	-	32	f5i14	at1g65520	enoyl-CoA hydratase/isomerase family,	PTS1		
3	1	2	3	37	Experimental	-	33	dt_d_20	at4g18360	glycolate oxidase - like protein	PTS1	15	
-	-	-	-	38	-	-	33	moa2	at3g14420	glycolate oxidase, putative	PTS1	15	
-	-	-	-	306	-	-	33	mln21	at3g14415	putative glycolate oxidase	PTS1	15	
-	-	-	-	54	-	-	34	k1o13	at5g41240	glutathione transferase	PTS1		
1	1	3	6	55	Experimental	-	34	mee6	at5g41210	glutathione transferase (AtGST 10)	PTS1		
-	-	-	-	116	-	-	35	vt_d_23	at4g37800	endo-xyloglucan transferase - like protein	PTS2		
1	2	3	11	146	Experimental	-	35	sa_d_27	at4g25820	xyloglucan endotransglycosylase XTR9	PTS1		
1	2	2	7	114	Experimental	-	36	t1j8	at2g36970	putative glucosyltransferase	PTS2		
-	-	-	-	142	-	-	36	f18o19	at2g43820	putative glucosyltransferase	PTS2		
1	2	1	11	102	Experimental	-	37	f23f1	at2g29990	putative NADH dehydrogenase	PTS1		
-	-	-	-	152	-	-	37	f10k1	at1g07180	NADH dehydrogenase family	PTS1		
1	1	5	11	285	Experimental	-	38	t18a20	at1g53850	20S proteasome alpha subunit E1 (PAE1)	PTS1		
-	-	-	-	299	-	-	38	mln21	at3g14290	20S proteasome subunit PAE2	PTS1		
1	1	1	3	3	Experimental	-	39	f11i4	at1g48640	pex3 like	-		
1	2	1	4	6	Experimental	-	40	myj24	at5g23050	acetyl-CoA synthetase-like protein	PTS1		
1	1	1	5	7	Experimental	-	41	f12c20	at2g26800	hydroxymethylglutaryl-CoA lyase putative	PTS1		
1	2	1	5	8	Experimental	-	42	mqn23	at5g65110	acyl-CoA oxidase (ACX2)	PTS2	31	
1	2	1	6	10	Experimental	-	43	dy_c_5	at3g51840	acyl-coA oxidase (ACX4)	PTS1	16	
3	1	1	4	15	Experimental	-	44	k7l4	at3g15290	3-hydroxybutyryl-CoA dehydrogenase putative	PTS1		
3	1	1	6	19	Experimental	-	45	mnl12	at5g43280	enoyl CoA hydratase-like protein	PTS1		
3	2	1	6	20	Experimental	-	46	f8a5	at1g60550	enoyl-CoA hydratase/isomerase Putative	PTS2		
1	1	2	2	23	Experimental	-	47	t1n6	at1g01710	acyl CoA thioesterase, putative	PTS1		
1	1	2	3	25	Experimental	-	48	f3e22	at3g06810	acetyl-coA dehydrogenase, putative	PTS1		
1	2	2	6	32	Experimental	-	49	sa_e_21	at5g03860	malate synthase -like protein	PTS1	17	
3	1	2	1	33	Experimental	-	50	msd21	at3g21720	isocitrate lyase putative	PTS1	18	
3	2	2	1	34	Experimental	-	51	f20d21	at1g54340	NADP specific isocitrate dehydrogenase	PTS1		
3	1	2	4	39	Experimental	-	52	t23k23	at1g68010	hydroxypyruvate reductase (HPR)	PTS1	19	
3	2	2	4	40	Experimental	-	53	f21o3	at3g07560	pex13 like	-		
1	1	3	1	45	Experimental	-	54	f14o4	at2g13360	alanine-glyoxylate aminotransferase (AGT1)	PTS1	20	
1	2	3	1	46	Experimental	-	55	dt_e_23	at5g11520	aspartate aminotransferase (Asp3)	PTS2	21	
1	1	3	4	51	Experimental	-	56	by_d_20	at4g35000	L-ascorbate peroxidase	-	22	
1	2	3	4	52	Experimental	-	57	mrg7	at5g18100	Cu/Zn superoxide dismutase-like protein	PTS1		
1	1	3	5	53	Experimental	-	58	tn_d_28	at4g31870	glutathione peroxidase - like protein	PTS1		
1	2	3	6	56	Experimental	-	59	mg_c_20	at3g52880	monodehydroascorbate reductase (NADH) - like protein	PTS1		
3	1	3	1	57	Experimental	-	60	f12k11	at1g06460	heat shock protein, putative	PTS1,2		
3	2	3	1	58	Experimental	-	61	pl_d_20	at4g18870	heat shock transcription factor - like protein	PTS1		

3	1	3	2	59	Experimental	-	62	k12b20	at5g37670	low-molecular-weight heat shock protein - like	PTS1	
3	2	3	2	60	Experimental	-	63	mah20	at5g08470	pex1 like	-	
3	1	3	3	61	Experimental	-	64	f19k16	at1g79810	pex2 like (TED3)	-	23
3	2	3	3	62	Experimental	-	65	sr_d__27	at4g29380	pex2 like	-	
3	2	3	4	64	Experimental	-	66	ch_e_25	at5g25760	pex4 like	-	
3	1	3	5	65	Experimental	-	67	mxk23	at5g56290	pex5	-	32
3	2	3	5	66	Experimental	-	68	f10o3	at1g03000	pex6 like	-	
3	1	3	6	67	Experimental	-	69	f28n24	at1g29260	pex7	-	32
3	2	3	6	68	Experimental	-	70	t9j22	at2g26350	pex10 like	-	
1	2	4	1	70	Experimental	-	71	t27c4	at3g04460	pex12 like	-	
1	1	4	2	71	Experimental	-	72	mqb2	at5g62810	pex14 (PED2)	-	24
1	2	4	2	72	Experimental	-	73	f4l18	at2g45690	pex16 (SSE1)	-	25
1	1	4	4	75	Experimental	-	74	my_d_45	at4g39850	peroxisomal ABC transporter (PED3/PXA1)	-	26,27
1	2	4	4	76	Experimental	-	75	f20d21	at1g54350	hypothetical peroxisomal half ABC transporter	-	
1	1	4	5	77	Experimental	-	76	t28m21	at2g39970	peroxisomal membrane carrier protein putative	-	
1	2	4	5	78	Experimental	-	77	my_e_21	at5g07320	peroxisomal Ca-dependent solute carrier-like protein	-	
1	1	4	6	79	Experimental	-	78	t26i20	at2g14860	PMP22	-	
1	2	4	6	80	Experimental	-	79	t26n6	at4g04470	PMP22	-	28
3	1	4	1	81	Experimental	-	80	f5k7	at2g06050	12-oxophytodienoate-10,11-reductase (OPR3)	PTS1	29
3	1	4	3	85	Experimental	-	81	k10d20	at3g20530	protein kinase, putative	PTS1	
3	2	4	3	86	Experimental	-	82	bt_d_20	at4g31220	protein kinase-like protein	PTS1	
3	1	4	4	87	Experimental	-	83	f23o10	at1g69270	receptor protein kinase -related	PTS1	
3	2	4	4	88	Experimental	-	84	ge_c_32	at3g61960	serine/threonine-protein kinase-like protein	PTS1	
3	1	4	5	89	Experimental	-	85	pl_d_20	at4g18950	protein kinase - like protein	PTS1	
-	-	-	-	90	-	-	86	k3g3	at3g25040	ER lumen retaining receptor (HDEL receptor), putative	PTS2	
3	1	4	6	91	Experimental	-	87	mjg19	at3g13220	ABC transporter	PTS1	
3	2	4	6	92	Experimental	-	88	t5m16	at1g77440	20S proteasome beta subunit PBC2 putative	PTS2	
1	2	1	7	94	Experimental	-	89	mqd22	at5g47040	mitochondrial Lon protease homolog 1 precursor	PTS1	
1	1	1	8	95	Experimental	-	90	t11a7	at2g41790	putative zinc protease	PTS1	
3	1	1	7	103	Experimental	-	91	bs_c_28	at3g56460	quinone reductase-like protein	PTS1	
3	2	1	7	104	Experimental	-	92	sa_d_23	at4g21490	NADH dehydrogenase like protein	PTS1	
3	2	1	8	106	Experimental	-	93	mhk10	at2g42490	putative copper amine oxidase	PTS1	
3	2	1	9	108	Experimental	-	94	t1d16	at2g26230	putative uricase subunit	PTS1	
3	1	1	10	109	Experimental	-	95	k16h17	at5g24400	6-phosphogluconolactonase-like protein	PTS1	
3	2	1	10	110	Experimental	-	96	tn_e_24	at5g04620	8-amino-7-oxononanoate synthase - like protein	PTS1	
3	1	1	11	111	Experimental	-	97	by_c_22	at3g52750	plastid division protein FtsZ -like	PTS1	
3	2	1	11	112	Experimental	-	98	f23o10	at1g69180	transcription factor CRC	PTS2	
1	1	2	8	115	Experimental	-	99	t7m13	at3g10700	galactokinase-like protein	PTS2	
1	2	2	9	118	Experimental	-	100	f16f14	at2g16440	putative CDC21 protein	PTS1	
1	1	2	10	119	Experimental	-	101	t23k3	at2g01880	putative purple acid phosphatase	PTS1	
1	2	2	10	120	Experimental	-	102	t3f17	at2g46020	putative SNF2 subfamily transcriptional activator	PTS1	
1	1	2	11	121	Experimental	-	103	fca_all	at4g15330	cytochrome P450 like protein	PTS1	

1	2	2	11	122	Experimental	-	104	mfo20	at5g42620	major surface glycoprotein-like	PTS1	
1	1	2	12	123	Experimental	-	105	f14j22	at1g49670	ARP protein, putative	PTS1	
1	2	2	12	124	Experimental	-	106	ge_c_25	at3G48170	betaine aldehyde dehydrogenase-like protein	PTS1	
3	1	2	7	125	Experimental	-	107	wt_d_26	at4g24550	clathrin coat assembly like protein	PTS1	
3	2	2	7	126	Experimental	-	108	tn_e_23	at5g04660	cytochrom P450 - like protein	PTS1	
3	1	2	8	127	Experimental	-	109	mpi7	at5g17890	disease resistance protein - like	PTS1	
3	1	2	9	129	Experimental	-	110	vt_d_22	at4g27760	forever young gene (FEY)	PTS1	
3	2	2	9	130	Experimental	-	111	t26f17	at1g21870	glucose 6 phosphate/phosphate translocator, putative	PTS1	
3	2	2	10	132	Experimental	-	112	k2k18	at5g35600	histone deacetylase	PTS1	
3	1	2	11	133	Experimental	-	113	dy_c_20	at3g51660	LS1-like protein	PTS1	
-	-	-	-	134	-	-	114	f6f3	at1g01290	molybdopterin biosynthesis CNX3 protein, putative	PTS1	30
3	1	2	12	135	Experimental	-	115	f10c21	at1g33440	nitrate transporter NTL1, putative	PTS1	
-	-	-	-	136	-	-	116	mca23	at5g47800	photoreceptor-interacting protein-like	PTS2	
1	2	3	7	138	Experimental	-	117	by_c_22	at3g52780	purple acid phosphatase -like protein	PTS1	
1	1	3	8	139	Experimental	-	118	t6a23	at2g38760	putative annexin	PTS1	
1	2	3	8	140	Experimental	-	119	t6b13	at2g14640	putative retroelement pol polyprotein	PTS2	
1	1	3	9	141	Experimental	-	120	f13k3	at2g36760	putative glucosyltransferase	PTS2	
1	1	3	10	143	Experimental	-	121	ch_e_41	at5g18830	squamosa promoter binding protein-like 7	PTS2	
1	2	3	10	144	Experimental	-	122	si_d_20	at4g13910	putative disease resistance	PTS2	
1	1	3	11	145	Experimental	-	123	sa_e_20	at5g02030	putative homeodomain protein	PTS2	
1	2	3	12	148	Experimental	-	124	f23m2	at2g11900	pseudogene	PTS2	
3	2	3	7	150	Experimental	-	125	f3f19	at1g13200	F-box protein family	PTS1	
-	-	-	-	151	-	-	126	t15m6	at1g58000	hypothetical protein	PTS1	
3	2	3	10	156	Experimental	-	127	f5m15	at1g20540	transducin / WD-40 repeat protein family	PTS1	
1	1	4	7	161	Experimental	-	128	f7o12	at1g76870	hypothetical protein	PTS1	
1	2	4	7	162	Experimental	-	129	f17f16	at1g16730	hypothetical protein	PTS1	
1	1	4	8	163	Experimental	-	130	f16n3	at1g47540	trypsin inhibitor 2 -related	PTS1	
1	2	4	8	164	Experimental	-	131	f20d21	at1g54390	expressed protein	PTS1	
1	1	4	9	165	Experimental	-	132	f20d21	at1g54540	hypothetical protein	PTS1	
1	2	4	10	168	Experimental	-	133	f18b13	at1g80060	hypothetical protein	PTS1	
1	1	4	11	169	Experimental	-	134	tle4	at1g33600	leucine rich repeat protein family	PTS1	
1	2	4	11	170	Experimental	-	135	f16m14	at2g38150	glycosyltransferase-related	PTS1	
1	1	4	12	171	Experimental	-	136	f4i1	at2g44400	CHP-rich zinc finger protein	PTS1	
-	-	-	-	172	-	-	137	t10j7	at2g12100	Ulp1 protease family	PTS1	
3	1	4	7	173	Experimental	-	138	f24l7	at2g32640	expressed protein\	PTS1	
3	2	4	7	174	Experimental	-	139	t1e2	at2g27890	hypothetical protein	PTS1	
3	1	4	8	175	Experimental	-	140	f14o13	at3g23860	hypothetical protein	PTS1	
3	2	4	8	176	Experimental	-	141	f26k24	at3g11840	expressed protein\	PTS1	
3	1	4	9	177	Experimental	-	142	t2e22	at3g12420	hypothetical protein	PTS1	
3	2	4	9	178	Experimental	-	143	mng15	at3g28020	hypothetical protein	PTS1	
3	1	4	10	179	Experimental	-	144	k17e7	at3g29790	hypothetical protein	PTS1	
3	2	4	10	180	Experimental	-	145	bs_c_29	at3g56870	hypothetical protein	PTS1	

3	1	4	11	181	Experimental	-	146	am_c_21	at3g61660	hypothetical protein	PTS1
3	2	4	11	182	Experimental	-	147	f11a12	at3g02360	6-phosphogluconate dehydrogenase -related	PTS1
3	1	4	12	183	Experimental	-	148	sa_d_29	at4g12380	hypothetical protein	PTS1
3	2	4	12	184	Experimental	-	149	vt_d_20	at4g19370	hypothetical protein	PTS1
1	1	5	1	185	Experimental	-	150	f6n23	at4g00470	hypothetical protein	PTS1
1	2	5	1	186	Experimental	-	151	t15b16	at4g01570	pentatricopeptide (PPR) repeat-containing protein	PTS1
1	1	5	2	187	Experimental	-	152	f21j9	at1g24530	transducin / WD-40 repeat protein family	PTS2
1	1	5	3	189	Experimental	-	153	f2k11	at1g63630	hypothetical protein	PTS2
1	2	5	4	192	Experimental	-	154	t12o21	at1g32020	F-box protein family	PTS2
1	1	5	5	193	Experimental	-	155	f15h11	at1g70920	homeodomain-leucine zipper protein	PTS2
1	2	5	6	196	Experimental	-	156	f28b23	at1g26230	chaperonin precursor, putative	PTS2
3	1	5	1	197	Experimental	-	157	fca_all	at4g16410	expressed protein	PTS2
-	-	-	-	199	-	-	158	t22c2	at3g31530	similar to polyprotein	PTS2
3	2	5	2	200	Experimental	-	159	f13m14	at3g10590	myb family transcription factor	PTS2
3	1	5	3	201	Experimental	-	160	myf24	at3g18500	expressed protein	PTS2
3	2	5	3	202	Experimental	-	161	f20c19	at3g26350	hypothetical protein	PTS2
3	1	5	5	205	Experimental	-	162	rr_c_22	at3g49590	expressed protein	PTS2
3	2	5	5	206	Experimental	-	163	dy_c_20	at3g51740	leucine-rich repeat transmembrane protein kinase	PTS2
3	2	5	6	208	Experimental	-	164	t1j24	at4g05530	short-chain dehydrogenase/reductase family protein	PTS1
1	1	6	1	209	Experimental	-	165	my_d_45	at4g39880	expressed protein	PTS2
1	2	6	1	210	Experimental	-	166	rr_c_28	at3g62230	F-box protein family	PTS2
1	1	6	2	211	Experimental	-	167	mx_c_24	at3g43170	hypothetical protein	PTS1
1	2	6	2	212	Experimental	-	168	po_c_22	at3g50170	hypothetical protein	PTS1
1	1	6	3	213	Experimental	-	169	me_c_21	at3g52280	hypothetical protein	PTS1
1	2	6	3	214	Experimental	-	170	po_c_20	at3g55040	ln2-1 protein	PTS1
1	1	6	4	215	Experimental	-	171	bs_c_21	at3g57810	auxin-regulated protein	PTS1
1	1	6	5	217	Experimental	-	172	ve_c_23	at3g59710	short-chain dehydrogenase/reductase family protein	PTS1
1	2	6	5	218	Experimental	-	173	ge_c_33	at3g60680	expressed protein	PTS1
1	1	6	6	219	Experimental	-	174	gl_c_20	at3g61200	thioesterase-related	PTS1
1	2	6	6	220	Experimental	-	175	wt_d_27	at4g23760	expressed protein	PTS1
3	2	6	1	222	Experimental	-	176	bs_d_21	at4g30830	expressed protein	PTS1
3	1	6	2	223	Experimental	-	177	dt_d_23	at4g35370	transducin / WD-40 repeat protein family	PTS1
3	2	6	3	226	Experimental	-	178	ku_e_25	at5g14900	helicase associated (HA2) domain-containing protein	PTS2
3	1	6	4	227	Experimental	-	179	pl_e_20	at5g01360	expressed protein	PTS1
3	2	6	4	228	Experimental	-	180	k21c13	at5g45000	disease resistance protein (TIR class)	PTS1
3	1	6	5	229	Experimental	-	181	k8a10	at5g67040	hypothetical protein	PTS1
3	2	6	5	230	Experimental	-	182	ch_e_26	at5g26270	hypothetical protein	PTS1
3	1	6	6	231	Experimental	-	183	ch_e_43	at5g34790	putative protein	PTS1
3	2	6	6	232	Experimental	-	184	dt_e_22	at5g09580	hypothetical protein	PTS1
1	2	7	1	234	Experimental	-	185	mjj3	at5g05660	expressed protein	PTS1
1	1	7	3	237	Experimental	-	186	mhk7	at5g40830	expressed protein	PTS1
1	2	7	3	238	Experimental	-	187	mbd2	at5g42890	expressed protein	PTS1

1	1	7	4	239	Experimental	-	188	mln1	at5g44250	expressed protein	PTS1
1	2	7	4	240	Experimental	-	189	mza15	at5g46670	CHP-rich zinc finger protein	PTS1
1	1	7	5	241	Experimental	-	190	mqd22	at5g46920	intron maturase	PTS1
1	1	7	6	243	Experimental	-	191	k24c1	at5g56220	expressed protein	PTS1
1	2	7	6	244	Experimental	-	192	mcd7	at5g56430	F-box protein family	PTS1
3	1	7	1	245	Experimental	-	193	en_e_21	at5g13070	expressed protein	PTS1
3	2	7	1	246	Experimental	-	194	my_e_25	at5g09330	expressed protein	PTS1
3	1	7	2	247	Experimental	-	195	sa_e_21	at5g03930	hypothetical protein	PTS1
3	2	7	2	248	Experimental	-	196	sa_e_24	at5g11210	glutamate receptor family (GLR2.5)	PTS1
3	1	7	3	249	Experimental	-	197	wr_c_20	at3g43140	hypothetical protein	PTS2
-	-	-	-	250	-	-	198	k11i1	at5g46500	expressed protein	PTS2
3	1	7	4	251	Experimental	-	199	ch_e_38	at5g18460	expressed protein	PTS2
-	-	-	-	254	-	-	200	wu_e_41	at5g32630	putative protein	PTS2
3	1	7	6	255	Experimental	-	201	wu_e_21	at5g19260	expressed protein	PTS2
3	2	7	6	256	Experimental	-	202	dt_e_26	at5g13250	hypothetical protein	PTS2
-	-	-	-	257	-	-	203	t10f5	at2g13590	pseudogene, Ta11-related non-LTR retroelement protein	PTS2
1	2	8	2	258	Experimental	-	204	f15h18	at1g18460	lipase family	PTS1
1	1	8	4	261	Experimental	-	205	f2p9	at1g73970	expressed protein	PTS1
1	2	8	4	262	Experimental	-	206	f3h9	at1g28320	expressed protein	PTS1
1	1	8	5	263	Experimental	-	207	t16b12	at2g31070	TCP family transcription factor	PTS1
1	2	8	5	264	Experimental	-	208	f16m14	at2g38180	GDSL-motif lipase/hydrolase protein	PTS1
1	1	8	6	265	Experimental	-	209	f16p2	at2g29590	thioesterase-related	PTS1
3	1	8	2	267	Experimental	-	210	msa6	at3g21070	expressed protein	PTS1
3	2	8	2	268	Experimental	-	211	k14a17	at3g17020	expressed protein	PTS1
3	2	8	3	270	Experimental	-	212	k20m4	at3g13610	oxidoreductase, 2OG-Fe(II) oxygenase family	PTS1
3	1	8	4	271	Experimental	-	213	mbk21	at3g12790	unknown protein	PTS1
3	2	8	4	272	Experimental	-	214	mjb21	at5g42670	expressed protein	PTS1
3	1	8	5	273	Experimental	-	215	mqo24	at5g43680	expressed protein	PTS1
3	2	8	5	274	Experimental	-	216	k23l20	at5g44780	expressed protein	PTS1
1	1	5	7	277	Experimental	-	217	f19b11	at2g03800	expressed protein	PTS2
1	2	5	7	278	Experimental	-	218	f3n11	at2g25730	expressed protein	PTS2
1	1	5	8	279	Experimental	-	219	t19l18	at2g25950	expressed protein	PTS2
1	2	5	8	280	Experimental	-	220	t9j23	at2g48030	endonuclease/exonuclease/phosphatase family	PTS2
3	1	5	11	297	Experimental	-	221	f17f8	at1g30990	low similarity to major latex protein	PTS2
3	2	5	11	298	Experimental	-	222	f17f8	at1g31060	expressed protein	PTS2
-	-	-	-	300	-	-	223	my_e_29	at5g08770	expressed protein	PTS1
-	-	-	-	301	-	-	224	sa_e_22	at5g04330	cytochrome P450, putative	PTS2

1	1	6	8	actin_2(ACT2)	Control	Internal
1	2	6	8	actin_2(ACT2)	Control	Internal
3	1	6	8	actin_2(ACT2)	Control	Internal
3	2	6	8	actin_2(ACT2)	Control	Internal
1	1	6	10	Arabidopsis_chlorophyll_ab_binding_protein	Control	Internal

1	2	6	10	Arabidopsis_chlorophyll_ab_binding_protein	Control	Internal
3	1	6	10	Arabidopsis_chlorophyll_ab_binding_protein	Control	Internal
3	2	6	10	Arabidopsis_chlorophyll_ab_binding_protein	Control	Internal
1	1	6	7	beta-6_tubulin(TUB6)	Control	Internal
1	2	6	7	beta-6_tubulin(TUB6)	Control	Internal
3	1	6	7	beta-6_tubulin(TUB6)	Control	Internal
3	2	6	7	beta-6_tubulin(TUB6)	Control	Internal
1	1	6	9	glyceraldehyde-3-phosphate_dehydrogenase(GapB)	Control	Internal
1	2	6	9	glyceraldehyde-3-phosphate_dehydrogenase(GapB)	Control	Internal
3	1	6	9	glyceraldehyde-3-phosphate_dehydrogenase(GapB)	Control	Internal
3	2	6	9	glyceraldehyde-3-phosphate_dehydrogenase(GapB)	Control	Internal
1	1	8	10	lambda-A	Control	External
1	2	8	10	lambda-A	Control	External
3	1	8	10	lambda-A	Control	External
3	2	8	10	lambda-A	Control	External
1	1	1	1	position_marker	Control	Positive
1	1	1	12	position_marker	Control	Positive
1	1	8	1	position_marker	Control	Positive
1	1	8	12	position_marker	Control	Positive
1	2	1	1	position_marker	Control	Positive
1	2	1	12	position_marker	Control	Positive
1	2	8	1	position_marker	Control	Positive
1	2	8	12	position_marker	Control	Positive
3	1	1	1	position_marker	Control	Positive
3	1	1	12	position_marker	Control	Positive
3	1	8	1	position_marker	Control	Positive
3	1	8	12	position_marker	Control	Positive
3	2	1	1	position_marker	Control	Positive
3	2	1	12	position_marker	Control	Positive
3	2	8	1	position_marker	Control	Positive
3	2	8	12	position_marker	Control	Positive
1	1	8	9	pUC19	Control	Negative
1	2	8	9	pUC19	Control	Negative
3	1	8	9	pUC19	Control	Negative
3	2	8	9	pUC19	Control	Negative

*-; the gene is not on array.

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Supplemental Data 2 Origin of DNA Fragments on the Peroxisome Specific Microarray and Primers Constructed for the Microarray or Quantitative RT-PCR

Sequence ID	Cluster#	Annotation	Biosequence Type*	Genbank	Clone ID	5' primer	3' primer	5' primer for quantitive RT-PCR	3' primer for quantitive RT-PCR
at5g27600	1	long-chain-fatty-acid-CoA ligase-like protein (acyl CoA synthetase)	cDNA clone	AV548579	RZL57d05F				
at3g05970	1	long-chain-fatty-acid-CoA ligase putative (acyl CoA synthetase)	cDNA clone	AV551395	RZ126b08R				
at5g16370	2	AMP-binding protein, putative (acyl CoA synthetase)	genomic clone			GAGTCGTACACTGCCACAGA	GAAGTTTTAGGCAGCTCATC		
at3g16910	2	AMP-binding protein putative (acyl CoA synthetase)	cDNA clone	AI100102	104H9XP (3')				
at1g20560	3	AMP-dependent synthetase and ligase family	cDNA clone	Z25500	GBGe60				
at1g66120	3	AMP-dependent synthetase and ligase family	genomic clone			ATGTTCCGTGTCCACAACG	GATGAGGCATGGCTACAACC		
at1g65880	3	AMP-dependent synthetase and ligase family	cDNA clone	BE524311	M48D9 (5')				
at1g65890	3	AMP-binding protein, putative	-						
at4g16760	4	acyl-CoA oxidase (ACX1)	cDNA clone	AV547491	RZL33c09F			TGGAGCAAGACATAGGTGGC	TACGAAGTTGCTGCTGAAGC
at2g35690	4	acyl-CoA oxidase putative	genomic clone			ACATTGCCTGAAGCTCATGC	CCGAGTCATTGAGTGGATCC	TGGTTAAACCCTGGTATGGTGT	AACGGTCGGATGTACTCACG
at1g06310	5	acyl-CoA oxidase like	genomic clone			GCAACACATGTCCTAACGTCC	AGATGATGTGAGTTGAGTCGG	CACTAAGATGCAGCCAGTTCC	CAAGTGCGTGAGGTCTAAGC
at1g06290+at1g0630	5	acyl-CoA oxidase (ACX3)	genomic clone			GCTATGGATTCGATGTCAAGG	GGTTGCATGCTGAACAACC	AGCAATCTGTTTAATCGGAAGG	CACAGTCAGCAATGCGGATA
at4g29010	6	fatty acid multifunctional protein (AIM1)	cDNA clone	AV529998	APZL55a10R			TGCCAAGGTCTATGGTGACC	CTCATTCCAAGGACAGACGC
at3g06860	6	fatty acid multifunctional protein (AtMFP2)	cDNA clone**					ACACACAGGCAGCTATGTTCC	TTCACCATAAGCCTTCGACC
at2g30650	7	3-hydroxyisobutyryl-coenzyme A hydrolase	genomic clone			CCAAAAAATCCAGGGAGTCTTGAC	GGGGACATTCCACCTATTGTTCG		
at2g30660	7	3-hydroxyisobutyryl-coenzyme A hydrolase	genomic clone			GCATGCAAGTTGTTCCTTGG	CGACTTCCGTTCCTAAGTTGG		
at5g65940	7	3-hydroxyisobutyryl-coenzyme A hydrolase (CHY1)	cDNA clone	AV551093	RZ121b04R				
at5g48880	8	3-ketoacyl-CoA thiolase (KAT5)	cDNA clone**					TGATCATATTCCTGGAGGCG	ACTAGCATTGCCAGCTGTGG
at2g33150	8	3-ketoacyl-CoA thiolase (PED1/ KAT2)	cDNA clone	AV543883	RZ26g09F			AGAAAGGCAGCTGCTGCTACT	CTCCTGCACCATCACTTACT
at1g04710	8	acetyl-CoA acyltransferase putative (KAT1)	cDNA clone	AV440397	APD61d07_f			AAGGCTGCTTCTGCTACT	ACAGAGCTCATCCACACCGC
at3g58740	9	citrate synthase -like protein	cDNA clone	AV562548	SQ171g06F				
at3g58750	9	citrate synthase -like protein	cDNA clone	AV533265	FB058g01F			GCTAGCTGATTGGGAGTTCA	GAGCAACTGCTACCTCGATG
at2g42790	9	citrate synthase putative	cDNA clone	AV534134	FB074e08F			TTCCTCTATATGCTTGATTCCC	GTGCAACAGCAACCTCAATT
at5g09660	10	malate dehydrogenase microbody NAD-dependent	cDNA clone**					ATGGAGTTTCGTGGAGATGC	TTTCTTGAAAACCTCAGCGG
at2g22780	10	malate dehydrogenase precursor putative glyoxysomal	cDNA clone	AV441698	APD15h07_r			TGCTCATCTCAATCCTCCTA	TCAGAGAGTGTCCTCACAATCC
at2g05710	11	cytoplamic aconitate hydratase	cDNA clone	AV528341	APZL06h08R				
at4g26970	11	putative aconitase	cDNA clone	AV545875	RZL03h03F				
at4g35830	11	aconitate hydratase	-						
at1g70580	12	putative alanine aminotransferase (AtGGT2)	cDNA clone	AV526259	APZ08e11R				
at1g23310	12	putative alanine aminotransferase (AtGGT1)	cDNA clone	AV520329	APZ16e05F				
at3g08860	13	putative aminotransferase	cDNA clone	AV557269	SQ064d07F			CGGAACGTCCGGTCAAG	CCTCTCACGTCTCCAATGAGT
at2g38400	13	beta-alanine-pyruvate aminotransferase putative (AGT3)	cDNA clone	AV521911	APZ68h03F				
at4g39660	13	alanin aminotransferase (AGT2)	cDNA clone	AV527682	APZ42e07R (5')				
at3g48730	14	glutamate-1-semialdehyde aminotransferase	cDNA clone	AV536777	pAZNII0530R				
at5g63570	14	glutamate-1-semialdehyde 2,1-aminomutase 1 precursor	cDNA clone	AV531984	FB033g09F				
at4g35090	15	catalase2	cDNA clone	AV538257	RZ112h09F				
at1g20620	15	catalase3	cDNA clone	AV565434	SQ224a03F				
at1g20630	15	catalase1	cDNA clone	AV554152	RZ82f08R (5')				
at3g47430	16	pex11 like	cDNA clone	AA713311	249E10T7				
at1g47750	16	pex11 like	cDNA clone**						
at3g03490	17	pex19 like	genomic clone			TCAAAGGGAAGCTCAAGAGG	ATTTGGCCAAGACTTGCGAAAT		
at5g17550	17	pex19 like	genomic clone			CAAAACTTAGTCACCTTGGGG	GGCACTTGATAAACTCAGGGAAC		

at3g48100	18	response reactor 2 (ATRR2)	cDNA clone	AV536923	pAZNII0702R					
at5g62920	18	response regulator 6 (ARR6)	cDNA clone	AV536542	pAZNII0260R					
at5g40310	19	zinc finger -like protein	genomic clone			CAACGAGAATCCTTGCTTTCCC	TTTGACTCCCTCCGTGAACATC			
at3g27970	19	exonuclease protein family	genomic clone			GATTGACGACTCGAATGGC	ATCAGGAGACATCCTCTCGG			
at4g05160	20	4-coumarate-CoA ligase - like protein	cDNA clone	T41627	62B7T7					
at4g19010	20	4-coumarate-CoA ligase - like	genomic clone			GCGCTCATCGATTCCTTAAC	AACATAGGTGGAACAACCGG			
at5g63380	20	4-coumarate-CoA ligase-like protein	cDNA clone	AV527088	APZ28d01R					
at1g20510	20	4-coumarate:CoA ligase 1 (4-coumaroyl-CoA synthase 1) (4CL1) fa	cDNA clone	AV547257	RZL28c05F					
at1g20500	20	4-coumarate:CoA ligase 1 (4-coumaroyl-CoA synthase 1) (4CL1) fa	cDNA clone	BE524101	M46A1 (5')					
at1g20490	20	4-coumarate:CoA ligase 1 (4-coumaroyl-CoA synthase 1) (4CL1) fa	cDNA clone	AV441457	APZ57h02_f					
at1g20480	20	4-coumarate:CoA ligase 1 (4-coumaroyl-CoA synthase 1) (4CL1) fa	-							
at5g08460	21	GDSL-motif lipase/acylhydrolase-like protein	cDNA clone	AV560568	SQ136g06F					
at3g04290	21	putative GDSL-motif lipase/acylhydrolase	cDNA clone	AV541758	RZ170g10F					
at2g19050	21	putative GDSL-motif lipase/hydrolase	genomic clone			GCAACGCCTACTTTGAACATTCC	CACATACCGTCTTTGAACTGGACAG			
at4g28780	21	Proline-rich APG - like protein	cDNA clone	AV555666	SQ021g11F					
at4g18970	21	GDSL-motif lipase/hydrolase protein	cDNA clone	AV541738	RZ170d12F					
at5g15720	21	GDSL-motif lipase/hydrolase protein	genomic clone			TTATGCCTGAGAGATACTCC	AAGGCATGTTAAGGCTCC			
at2g43020	22	putative amine oxidase	cDNA clone	AV526308	APZ09g11R					
at1g65840	22	polyamine oxidase, putative	cDNA clone	AV553038	RZ52b07R			TTGGCCTAATGTGGAGTTCC	TATCAACCGGCTCTCCTAGC	
at3g59050	22	similar to polyamine oxidase	cDNA clone	AV551630	RZ129d03R					
at2g43910	23	putative methyl chloride transferase	genomic clone			AGTCCTGAACGCTTCGTTGT	TGGAATGGCGTGAGGATTCT	TCCGAAAGCGCACTCGCG	TACGAGTTGGAATGGCGTGA	
at2g43920	23	thiol methyltransferase	cDNA clone	T04694	11D5T7P					
at4g01190	24	putative phosphatidylinositol kinase	genomic clone			TGATTGGAAGGATTACTGTCCC	GGATTTTTTACTTGGAGGTGCG			
at1g01460	24	4,5 PIP kinase -related	genomic clone			TGGTGTGAGCAGACATATT	TCTCACACCGTAGTCTTGG			
at1g50510	24	expressed protein	cDNA clone	AV558979	SQ109b02F					
at1g48320	25	thioesterase family	cDNA clone	AV543483	RZ201d08F					
at5g48950	25	thioesterase-related	genomic clone			CCTAACTGAAAAATGTTGCCAGCC	GGTAAACCGCAGAAGAGAGTAACCC			
at1g31710	26	copper amine oxidase -related	genomic clone			GAATATATTCACACATCCGA	AACCGACGGTGTACCACAT	ACACATCCGAAATCAAACTA	AACCGACGGTGTACCACAT	
at1g31690	26	copper amine oxidase -related	genomic clone			AATGACGACTGGTACTTC	ACGGTGTGATCCACACGTTG			
at3g42490	27	hypothetical protein	-							
at5g36070	27	hypothetical protein	genomic clone			CTCAACCAATGGCACCTACC	GACCAACACTCACCGTAATCC			
at1g35070	27	hypothetical protein	genomic clone			CTTTGCTATCTGAACAATCACCCG	CCGACCAACACTCACCGTAATC			
at4g39140	28	expressed protein	cDNA clone	AV563043	SQ180e06F					
at2g21500	28	expressed protein	cDNA clone	AV552726	RZ37a02R					
at5g33340	29	hypothetical protein	genomic clone			AGTTGATCCTCTCTTTGACC	AGGAGTCAAGCTTCACATCG			
at5g33350	29	putative protein	genomic clone			ATGGCGGCCGCTGACAC	CGATTCTTTCATCTTTCGACC			
at5g53840	30	F-box protein family	genomic clone			ACTTTCAGTTCTGTTCCACATCCC	TTGTTTCCTTGCCTTGCCTG			
at5g22660	30	F-box protein family	genomic clone			TACCGACATTGCTTCTCTCG	TGTGGACAATCATACCGTGG			
at1g60400	30	F-box protein family	genomic clone			TGAACTCTCCACCAAGGATTCG	AGTGATAGCCACGGATGACCTG			
at1g60410	30	F-box protein family	genomic clone			ATGAGCACTTGCATGAGCC	GAGCAGAGATGGTCAGTTCG			
at1g28710	31	expressed protein	genomic clone			GACTTCCTACGATCGTTGCC	CCAATTGCTGTTGACATGG			
at1g28700	31	hypothetical protein common family	genomic clone			GCCGTTGCTCTTCTATTTGCC	GAGGGAGATGGTCTTGTTGTTGG			
at5g40900	31	hypothetical protein common family	genomic clone			CGAGAATGGGCAAAACCTGAC	GGACTTGGCATAGAAGAAACCTCC			
at4g14430	32	carnitine racemase like protein	-							
at1g65520	32	enoyl-CoA hydratase/isomerase family,	cDNA clone	AV533834	FB068g09F					

at4g18360	33	glycolate oxidase - like protein	cDNA clone	AV545143	RZ73d05F			GATTCGCGCTTCCTCGAGGT	CCGCTGCAAGCGAGAACAAG
at3g14420	33	glycolate oxidase, putative	-					TCGTGAGAAGAGCTGAGAGG	GTGGTTACGGGAGATTTCCT
at3g14415	33	putative glycolate oxidase	-					GCTCCAAACAATCACCAACA	CTCCTTCAGCAGCTAGTGCA
at5g41240	34	glutathione transferase	-						
at5g41210	34	glutathione transferase (AtGST 10)	cDNA clone	AV552102	RZ20g05R				
at4g37800	35	endo-xyloglucan transferase - like protein	-						
at4g25820	35	xyloglucan endotransglycosylase XTR9	cDNA clone	AV542095	RZ178b02F				
at2g36970	36	putative glucosyltransferase	genomic clone			CGTTATCCCTTTTGTCCACTTAGC	ACATTCGCTGAGACTTGGTCCC		
at2g43820	36	putative glucosyltransferase	-						
at2g29990	37	putative NADH dehydrogenase	genomic clone			ATGACTCGAGTCAGGTACGC	CATGCTCAGGAGATTCGTAGG		
at1g07180	37	NADH dehydrogenase family	-						
at1g53850	38	20S proteasome alpha subunit E1 (PAE1)	cDNA clone	AV539667	RZ137a08F				
at3g14290	38	20S proteasome subunit PAE2	-						
at1g48640	39	pex3 like	cDNA clone	AV562462	SQ170e07F (3')				
at5g23050	40	acetyl-CoA synthetase-like protein	cDNA clone	AV523598	APZL33c09F				
at2g26800	41	hydroxymethylglutaryl-CoA lyase putative	cDNA clone	AV551110	RZ121d01R				
at5g65110	42	acyl-CoA oxidase (ACX2)	cDNA clone	AV558388	SQ097b11F				
at3g51840	43	acyl-coA oxidase (ACX4)	cDNA clone	AV534736	FB085b01F				
at3g15290	44	3-hydroxybutyryl-CoA dehydrogenase putative	cDNA clone	AV550666	RZ115f10R				
at5g43280	45	enoyl CoA hydratase-like protein	cDNA clone	AV552030	RZ19c07R				
at1g60550	46	enoyl-CoA hydratase/isomerase Putative	genomic clone			CAGCATCAAAACTTCCAACCTGC	CGTCATCCTATCCAATGGCG		
at1g01710	47	acyl CoA thioesterase, putative	cDNA clone	AV531734	FB029f06F				
at3g06810	48	acetyl-coA dehydrogenase, putative	cDNA clone	AA597367	196K19T7				
at5g03860	49	malate synthase -like protein	genomic clone			CGTTCCACGACAAGTCAAGG	TGGACACGGCTTATCTCAGC		
at3g21720	50	isocitrate lyase putative	cDNA clone	BE524944	M56G10 (5')			CATGAACCTCAACGAGAACG	GATTGTAGGCGAGCATGACC
at1g54340	51	NADP specific isocitrate dehydrogenase	genomic clone			GCCAATCTGTATTGGAAGGC	AGAGGCTATGCTGTTGGTGC		
at1g68010	52	hydroxypyruvate reductase (HPR)	cDNA clone	AV565220	SQ219g03F			GTCAACAAGGAGAGGCTTGC	TCAAGAATGGATCGACTCGG
at3g07560	53	pex13 like	cDNA clone	AV519033	APD46f07F (3')				
at2g13360	54	alanine-glyoxylate aminotransferase (AGT1)	cDNA clone	AV520266	APZ12h10F				
at5g11520	55	aspartate aminotransferase (Asp3)	cDNA clone	AV440535	APD67c12_f				
at4g35000	56	L-ascorbate peroxidase	cDNA clone	AV550316	RZ110g02R				
at5g18100	57	Cu/Zn superoxide dismutase-like protein	genomic clone			ATGGAAGCTCCTAGAGGAAA	TAGTTTAGCATCCGCAGATGATT		
at4g31870	58	glutathione peroxidase - like protein	genomic clone			CTTACGCATCATTCTCTACA	GAATTTGGTACCTCGATTTGG		
at3g52880	59	monodehydroascorbate reductase (NADH) - like protein	cDNA clone	AV521596	APZ61h08F				
at1g06460	60	heat shock protein, putative	cDNA clone	AV441099	APZ27f10				
at4g18870	61	heat shock transcription factor - like protein	genomic clone			GATAGTTTTGTATTCTCTC	CGTAGTTGATCATTCTTC		
at5g37670	62	low-molecular-weight heat shock protein - like	genomic clone			GTCTCGACTTCTGTAATCTG	ATACTGGCATACTGCAATTC		
at5g08470	63	pex1 like	cDNA clone	AV522725	APZL04c10F				
at1g79810	64	pex2 like (TED3)	cDNA clone**						
at4g29380	65	pex2 like	cDNA clone	AV547697	RZL37h02F				
at5g25760	66	pex4 like	cDNA clone	AV566071	SQ236b02F				
at5g56290	67	pex5	cDNA clone**						
at1g03000	68	pex6 like	cDNA clone	AV529457	APZL38b10R				
at1g29260	69	pex7	cDNA clone	AV528106	APZ50h10R				
at2g26350	70	pex10 like	genomic clone			ATGAAGCTACTTGGACAGATGC	CTCCTGAAGTTTGATAAGATCC		

at3g04460	71	pex12 like	cDNA clone	AV565895	SQ232f03F				
at5g62810	72	pex14 (PED2)	cDNA clone	AV542465	RZ184f04F				
at2g45690	73	pex16 (SSE1)	cDNA clone**						
at4g39850	74	peroxisomal ABC transporter (PED3/PXA1)	cDNA clone	AV442696	APZ31b03_r				
at1g54350	75	hypothetical peroxisomal half ABC transporter	cDNA clone	AV566729	SQ249c05F				
at2g39970	76	peroxisomal membrane carrier protein putative	cDNA clone	AV564541	SQ206b02F				
at5g07320	77	peroxisomal Ca-dependent solute carrier-like protein	genomic clone			ATCATCATTGGGAAAGGGTGTG	CTCTTGCTTCATTGTCGTCTTCG		
at2g14860	78	PMP22	genomic clone			TGAACCAGTAGTGAAGTGTTGGACC	TCTCGGAAGCCATAATGAGCAG		
at4g04470	79	PMP22	genomic clone			GCAATAACTGCTGGAGTTTTG	CCAAAGCTAGTGTCATTGACC		
at2g06050	80	12-oxophytodienoate-10,11-reductase (OPR3)	cDNA clone	AV558591	SQ102e04F				
at3g20530	81	protein kinase, putative	genomic clone			CAGCTAGACAGGAATGGTTACC	GAAGACACATTGCTGCTACTGC		
at4g31220	82	protein kinase-like protein	genomic clone			TTGGTCCGGAGCTGTTAATG	ATGGCTAATGGGAGCTTAGA		
at1g69270	83	receptor protein kinase -related	genomic clone			GAGAGTTTCGTTTCCATTTCCTCG	TCTGGGTTTGGTCTTCTTGCTG		
at3g61960	84	serine/threonine-protein kinase-like protein	cDNA clone	Z37594	FAI327 (5')				
at4g18950	85	protein kinase - like protein	genomic clone			TGAGGATGGAGAAGAGATCG	TGCCATACAGTAAGTTCCC		
at3g25040	86	ER lumen retaining receptor (HDEL receptor), putative	-						
at3g13220	87	ABC transporter	genomic clone			GGTACAAGACAGACTTGGAGCC	CACAGTTCTTGTAAGCCGCC		
at1g77440	88	20S proteasome beta subunit PBC2 putative	cDNA clone	AV558340	SQ095d12F				
at5g47040	89	mitochondrial Lon protease homolog 1 precursor	cDNA clone	AV530002	APZL55e03R				
at2g41790	90	putative zinc protease	cDNA clone	AV562337	SQ168e02F				
at3g56460	91	quinone reductase-like protein	cDNA clone	AV550893	RZ118h03R				
at4g21490	92	NADH dehydrogenase like protein	cDNA clone	T41616	62A4T7				
at2g42490	93	putative copper amine oxidase	cDNA clone	AV524364	APZL64f08F				
at2g26230	94	putative uricase subunit	cDNA clone	AV562981	SQ179d03F				
at5g24400	95	6-phosphogluconolactonase-like protein	genomic clone			GGTTGACGAGAGAGTTGTTCC	TGTGCAGATGAGTTGATGAC		
at5g04620	96	8-amino-7-oxononanoate synthase - like protein	cDNA clone	AV567301	SQL10g08F				
at3g52750	97	plastid division protein FtsZ -like	genomic clone			ATCAGATATTCCACGGACACC	CCTGATCATCATCTAGGCACG		
at1g69180	98	transcription factor CRC	cDNA clone	AV532867	FB051d08F				
at3g10700	99	galactokinase-like protein	cDNA clone	AV557154	SQ061g04F				
at2g16440	100	putative CDC21 protein	cDNA clone	AV556981	SQ057g11F				
at2g01880	101	putative purple acid phosphatase	cDNA clone	T42810	116P10T7			TCACGGTGTGACTCAAGAGC	GATCGGTGAAGAACATTGCC
at2g46020	102	putative SNF2 subfamily transcriptional activator	cDNA clone	AV528359	APZL07d01R				
at4g15330	103	cytochrome P450 like protein	cDNA clone	T21786	98D6T7				
at5g42620	104	major surface glycoprotein-like	cDNA clone	AV529248	APZL31h04R				
at1g49670	105	ARP protein, putative	genomic clone			CCGTGCACCACTACAATTACC	CCAATCACGATGAGTCGTCC		
at3G48170	106	betaine aldehyde dehydrogenase-like protein	cDNA clone	AV441863	APD10c03_r				
at4g24550	107	clathrin coat assembly like protein	cDNA clone	T22309	103P10T7				
at5g04660	108	cytochrom P450 - like protein	genomic clone			GAACATGCTTAGCTCAACACG	ATCAATCTCACTTCCTGGTGG		
at5g17890	109	disease resistance protein - like	cDNA clone	N96493	F2E10T7				
at4g27760	110	forever young gene (FEY)	cDNA clone	BE530722	600040295R1 (5')				
at1g21870	111	glucose 6 phosphate/phosphate translocator, putative	genomic clone			GTCATTGGTGCCTATTGTTGG	GAATGTGACTGCTGTTGTGG		
at5g35600	112	histone deacetylase	genomic clone			CTGTAAGCAATCACCATGACC	TAACCGATCCTCATCCTTCC		
at3g51660	113	LS1-like protein	genomic clone			GTTTAGAAGGAAGAGGC	GCAAACAAAGCTTAACGGGC		
at1g01290	114	molybdopterin biosynthesis CNX3 protein, putative	-						
at1g33440	115	nitrate transporter NTL1, putative	genomic clone			GGTCGAGACAAGAACAGAGC	ATCAAGACGCAAGGCAAGGC		

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at5g47800	116	photoreceptor-interacting protein-like	-						
at3g52780	117	purple acid phosphatase -like protein	genomic clone			ATGAGTCGCATTCTCGACC	TTGAAGAGAGTGGCTCGAGC		
at2g38760	118	putative annexin	genomic clone			TCTCAAACAAGCTATTCGCG	TGTTCCAAAACCCTCAATCG	TCTATCCTCCGAACTGTCTGG	TGGCTTCACGTAGCATAGCC
at2g14640	119	putative retroelement pol polyprotein	genomic clone			TTCATGGCATACCTCGTTCC	CATATCTGGCTCACCATCTCC		
at2g36760	120	putative glucosyltransferase	genomic clone			GCCAAGTGATCAATGGAACC	AGTCAGGCTTGCACATTAGG		
at5g18830	121	squamosa promoter binding protein-like 7	cDNA clone	AV528347	APZL07a09R				
at4g13910	122	putative disease resistance	genomic clone			TTGAGTTTCCCCAAGTTGCG	TGCTTCTTCATCTTCTTCTCGGC		
at5g02030	123	putative homeodomain protein	cDNA clone	AV565147	SQ218d01F				
at2g11900	124	pseudogene	genomic clone			CCTCCAAATCCGACCGAATC	CGAAAACTCAATCTTCTCCTCTGTAAG		
at1g13200	125	F-box protein family	genomic clone			GCACATGTCAATATGGTGAGG	AGATCGACGATCGAATCTCG		
at1g58000	126	hypothetical protein	-						
at1g20540	127	transducin / WD-40 repeat protein family	cDNA clone	AV553412	RZ62a11R				
at1g76870	128	hypothetical protein	genomic clone			GGAAAACTCTCCTTGGCAACG	CTCTCTTCTCTTACTAAACACCTCCCAC		
at1g16730	129	hypothetical protein	genomic clone			AAGCAGTGTCGGACGATGTG	AGATGTCGGAGAAACAAACGG		
at1g47540	130	trypsin inhibitor 2 -related	cDNA clone	Z47386	PAP1025				
at1g54390	131	expressed protein	cDNA clone	AV441648	APD12f10_r				
at1g54540	132	hypothetical protein	genomic clone			TTGTTGCTGCTGCAATGC	ATTCCGCCTCCTGTTATTCC		
at1g80060	133	hypothetical protein	cDNA clone	N65895	240L16T7				
at1g33600	134	leucine rich repeat protein family	cDNA clone	AV441668	APD15b11_r				
at2g38150	135	glycosyltransferase-related	genomic clone			TCGTCCATCTCTTGGTTGC	GATCCGTTGTTACCTCCTCG		
at2g44400	136	CHP-rich zinc finger protein	genomic clone			AAATACCAAGAAGGGCGATTCC	CGGCAACATCAAAGGATACGG		
at2g12100	137	Ulp1 protease family	-						
at2g32640	138	expressed protein\	cDNA clone	BE522111	M23G10 (5')				
at2g27890	139	hypothetical protein	cDNA clone	AV554919	RZL04g05R				
at3g23860	140	hypothetical protein	genomic clone			AACAAAGTCGGCAGTTTCG	CAAGAAGATAAGTGCTGTGGTTCC		
at3g11840	141	expressed protein\	genomic clone			TTGATGTGTGAATGTGGCG	CACAATCCATTCCTAGAACGG		
at3g12420	142	hypothetical protein	genomic clone			TTATCCGTCACCGTAACTTCG	CGTGAACATCAATTGACGC		
at3g28020	143	hypothetical protein	genomic clone			AGGATGTTCACTTCCTCATGG	CGGACATCATTCAACTCTTCG		
at3g29790	144	hypothetical protein	genomic clone			CATACAAGCGATGGTCTTGAG	ACCACCACACACCACACTTC		
at3g56870	145	hypothetical protein	genomic clone			GTGGTAGCCATTGAGGAAGC	AGACATGTTGGAGGTGTTGC		
at3g61660	146	hypothetical protein	genomic clone			CGAGCTCGTTTCTCTTACTC	AGAGAGGATCCGACAAAAC		
at3g02360	147	6-phosphogluconate dehydrogenase -related	cDNA clone	AV551212	RZ122g04R				
at4g12380	148	hypothetical protein	genomic clone			TTACAAGCGGCTGATGCTC	CCTTGGCAACGAACTGATTAC		
at4g19370	149	hypothetical protein	genomic clone			CGTGTTCTCTCTTGGTCTCG	TGGAAGACACTTCTTCCACC		
at4g00470	150	hypothetical protein	genomic clone			CCAACAGCTTCTGTAGTGAAGC	CCATCACCTGAACAACTCACC		
at4g01570	151	pentatricopeptide (PPR) repeat-containing protein	cDNA clone	AV552716	RZ36g05R				
at1g24530	152	transducin / WD-40 repeat protein family	cDNA clone	BE521747	M21C6 (3')				
at1g63630	153	hypothetical protein	genomic clone			TCTACTGCCATCGTTGATCG	CTGCAGATCCTCCAATATTGC		
at1g32020	154	F-box protein family	genomic clone			CCAGATCATTTGGTAGCTAAG	CTGTACTATAGACTGTCCTTGC		
at1g70920	155	homeodomain-leucine zipper protein	genomic clone			ATGAGAGATTTTGACATAAACC	GAATGATGTAATATATGAAGAGG		
at1g26230	156	chaperonin precursor, putative	cDNA clone	AV559035	SQ110a05F				
at4g16410	157	expressed protein	cDNA clone	AV535519	FB097f10F				
at3g31530	158	similar to polyprotein	-						
at3g10590	159	myb family transcription factor	genomic clone			AACGCAGGAAGAAGGATACG	GACGGATCATCATCATCCG		
at3g18500	160	expressed protein	genomic clone			CAAGCGAAGCTTACCGTATC	GACGTTATCTCCAGTTCGCC		

at3g26350	161	hypothetical protein	genomic clone			AACCACGCAACCTCACCCA	ATTCAATGAAACGCTGCAAT		
at3g49590	162	expressed protein	cDNA clone	AV558869	SQ107c03F				
at3g51740	163	leucine-rich repeat transmembrane protein kinase	cDNA clone	AV523399	APZL26e07F				
at4g05530	164	short-chain dehydrogenase/reductase family protein	cDNA clone	AV555954	SQ031b11F				
at4g39880	165	expressed protein	cDNA clone**						
at3g62230	166	F-box protein family	genomic clone			AGATTACCAGTCTCGTGACG	TATCAGCTGCAGCACGTATCG		
at3g43170	167	hypothetical protein	genomic clone			ATGTCCTCATCTTCTCCCGA	CAACTTGGAAGGTGTACGAC		
at3g50170	168	hypothetical protein	genomic clone			AACTTGGTACTTGCTCAGCC	CTAGACTGGAGTAGACTTCG		
at3g52280	169	hypothetical protein	genomic clone			GGAGGCTAAGGATGGTACCG	GTGCTCCACTCAATTCTCTA		
at3g55040	170	In2-1 protein	genomic clone			CAACGCTTGCGTTATCCTCG	CTATCTTGTTTTGCAGACCC		
at3g57810	171	auxin-regulated protein	genomic clone			ATGATGATTTGTTACTCTCCAAT	CCTGGTACGTAATTCGTCAG		
at3g59710	172	short-chain dehydrogenase/reductase family protein	cDNA clone	AV549204	RZ04g04R				
at3g60680	173	expressed protein	cDNA clone	AV540415	RZ15h10F				
at3g61200	174	thioesterase-related	cDNA clone	AA585756	104K17XP (3')				
at4g23760	175	expressed protein	genomic clone			ATGCAGGGAAAAGAGCTGAC	AGTAAGAAGTCAAACATCCGTTGAA		
at4g30830	176	expressed protein	genomic clone			AGCTGAGCTAGCAATGGAAGC	TCAACTCTCTGTCTCAACCG		
at4g35370	177	transducin / WD-40 repeat protein family	genomic clone			ATGCTCAAGTTACTCATGCC	CTTTGAGAGGACAATCAAGCC		
at5g14900	178	helicase associated (HA2) domain-containing protein	genomic clone			AGATTCTCCTGCTCCTGATACC	ATTGGAAGGATGCAAGTGC		
at5g01360	179	expressed protein	genomic clone			TTGGTGGATGAGTGGTCTCC	GTTGATGACTGTCACATGCG		
at5g45000	180	disease resistance protein (TIR class)	genomic clone			TGAGCCTCTAATGGCAGAGC	CAGTAGATGACTTCAAGG		
at5g67040	181	hypothetical protein	genomic clone			ATGGGTTCACTGCATCTCAA	GAGATGAGAAGGGATCCAGT		
at5g26270	182	hypothetical protein	genomic clone			TCGAGTCTAGATCTAGATCTA	GATTCTTGGAAGACTGTATCCA		
at5g34790	183	putative protein	genomic clone			AGGCGTGGGATGAGCAGGTG	GCCAAACCGTTCCATTGTTG		
at5g09580	184	hypothetical protein	genomic clone			CCAGGCACTCTCCATATAAC	TTGATCATCGGAACTCCG		
at5g05660	185	expressed protein	cDNA clone	AV538818	RZ121g04F				
at5g40830	186	expressed protein	cDNA clone	AV442505	APZ21a04_r				
at5g42890	187	expressed protein	genomic clone			ATGGCGAATACCCAACTCAA	CAACTTTGAAGGTTTAGGGAAG		
at5g44250	188	expressed protein	cDNA clone	AV566315	SQ241b03F				
at5g46670	189	CHP-rich zinc finger protein	genomic clone			TGTGCGCTTCCAATCTACG	AGTGGTGCAGCAGTCATTGC		
at5g46920	190	intron maturase	genomic clone			GGAGGTCATATCTTGATTGG	CCAACATCGATGTGTAATCC		
at5g56220	191	expressed protein	cDNA clone	AV541518	RZ167h12F				
at5g56430	192	F-box protein family	genomic clone			CTTCACCACATCCTTCTCTTCTTG	GCTTCTGGAAAGTCTTACCTCGTC		
at5g13070	193	expressed protein	cDNA clone	AV549314	RZ06b09R				
at5g09330	194	expressed protein	cDNA clone	AV564907	SQ213b03F				
at5g03930	195	hypothetical protein	genomic clone			GGAAGACGAGGCGGTAGTTAGAATC	GCAAGGAGGAAGAGAAAGGCAG		
at5g11210	196	glutamate receptor family (GLR2.5)	genomic clone			TCACCAGGCCATATTATCGG	TCAAGTCCAAGCCATTAGCG		
at3g43140	197	hypothetical protein	genomic clone			CTTGATTCGTTGTGATCGAT	CGAGTGCTTAAGAGTGAAGTAC		
at5g46500	198	expressed protein	-						
at5g18460	199	expressed protein	genomic clone			GAGTGATTCATATCAAGC	ATATTCAATCTTTCACTACC		
at5g32630	200	putative protein	-						
at5g19260	201	expressed protein	cDNA clone	AV554200	RZ83h08R			TGTTCTCGCTTGACGTGCA	GGTCAGCTTGAAAGCATCCG
at5g13250	202	hypothetical protein	genomic clone			CTGCTGCTGAATCCTCTTCC	ACCAATCATATCCTGAGGCC		
at2g13590	203	pseudogene, Ta11-related non-LTR retroelement protein	-						
at1g18460	204	lipase family	cDNA clone	AV522584	APZ83b12F				
at1g73970	205	expressed protein	cDNA clone	Z26071	GBGe216				

at1g28320	206	expressed protein	cDNA clone	H77159	204G17T7					
at2g31070	207	TCP family transcription factor	cDNA clone	AV442146	APZ39a05_r					
at2g38180	208	GDSL-motif lipase/hydrolase protein	genomic clone			CCTCATCCTTCAGGTCATGG	CGTTTTGGGTTAACTCATGGTCAC			
at2g29590	209	thioesterase-related	genomic clone			CAATTGAGCGATGAGGTGG	CGACCTTCCGCTATAATCTCC			
at3g21070	210	expressed protein	genomic clone			CATCTCGTTGAGTTCTCTGAGG	ACTACTGCGCTCTGCTGTGC			
at3g17020	211	expressed protein	cDNA clone	AV531000	FB013c02F					
at3g13610	212	oxidoreductase, 2OG-Fe(II) oxygenase family	cDNA clone	AV550272	RZ110b06R			TCAAGGCTGCGACTCACAAG	GATCACAAAAGATCCAGCAACC	
at3g12790	213	unknown protein	cDNA clone**							
at5g42670	214	expressed protein	cDNA clone	BE529375	600038652R1 (5')					
at5g43680	215	expressed protein	genomic clone			AATGCTTGGGCTTTCTTACGG	AATGATGTTGCTTGAGCGTGC			
at5g44780	216	expressed protein	cDNA clone**							
at2g03800	217	expressed protein	cDNA clone	AV565382	SQ223a03F					
at2g25730	218	expressed protein	cDNA clone	AV530133	APZL59g06R					
at2g25950	219	expressed protein	genomic clone			GCTCTGAATGAATCTGTTTCGGG	CGTATTTGGGTTGTGTCACCTCC			
at2g48030	220	endonuclease/exonuclease/phosphatase family	cDNA clone	AV550039	RZ107d08R					
at1g30990	221	low similarity to major latex protein	genomic clone			TCATCCGAATCAGCAATCAT	ATCGGCCGAGAAACACTACA	GGAGCTTAAGGTGTATATCCCAAA	TGGAGAATGTGGTCATCCGA	
at1g31060	222	expressed protein	genomic clone			ACTCTGCATGTTGTCCTGTCC	GAGAAGATACTACATGGTCGCG			
at5g08770	223	expressed protein	-							
at5g04330	224	cytochrome P450, putative	-							

*-; the gene is not on array.

**isolated in our laboratory

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Original Paper

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Other Publication

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