Degradation of plant peroxisomes by autophagy

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Peroxisomes play a critical role in many metabolic pathways during the plant life cycle. It has been proposed that the transition between different types of peroxisomes involves the degradation of obsolete peroxisomal enzymes via proteolytic activities in the peroxisome matrix, the cytosol, or the vacuole. Forward and reverse genetic studies recently provided evidence for autophagic degradation of peroxisomes in the vacuole of Arabidopsis seedlings. Here, we briefly review a model of pexophagy, or selective autophagy of peroxisomes, in plant cells.

Keywords: autophagy-related, Atg, peroxisome-associated protein degradation, hydrogen peroxide, glyoxylate cycle enzymes

PLANT Peroxisomes: TYPES, TRANSITION, AND PROTEIN DEGRADATION

Plant peroxisomes are versatile organelles that participate in many metabolic pathways such as fatty acid β-oxidation and photorespiration (reviewed by Hu et al., 2012). In addition to the enzymes needed for these pathways, peroxisomes contain antioxidant enzymes, for example, catalase, to protect plants from oxidative damage, since hydrogen peroxide is generated from fatty acid β-oxidation and photorespiration and other oxidation reactions in the peroxisome.

Peroxisomes are dynamic organelles with the capacity to change their appearance, their association with other organelles, and their enzyme composition. These changes depend on the developmental program and metabolic needs of the cell. For example, when oil-storing seeds such as cucumber (Cucumis sativus) and Arabidopsis (Arabidopsis thaliana) germinate, peroxisomes contain the glyoxylate cycle enzymes. The enzymes are needed for the consumption of acetyl-CoA (the product of fatty acid β-oxidation) for synthesis of organic acids that can be used to generate sugars by gluco-oxidation and photorespiration (reviewed by Pracharoenwattana and Smith, 2008).

When seedling peroxisomes are transformed to leaf peroxisomes, obsolete ICL and MLS must be degraded. In recent studies, three mechanisms have been proposed for the degradation of these proteins during post-germinative growth of Arabidopsis seedlings (Figure 1). In one mechanism (herein designated intraperoxisomal degradation (IPD)), it is proposed that peroxisomal proteins are degraded by resident proteases. However, known peroxisomal proteases, which include Lon-related protease 2 (LON2), have not been implicated in full degradation of peroxisomal matrix proteins (Lingard and Bartel, 2009). This argument was mainly based on the observation that ICL and MLS levels in lon2 mutant were not higher than those in wild-type (Lingard and Bartel, 2009; Burkhart et al., 2013). According to a second mechanism, obsolete proteases are retranslocated from peroxisomes and degraded in the cytosol by the 26S proteasome. During this process, called pexisome-associated protein degradation (PexAD), the proteins are polyubiquitylated before they are recognized by the proteasome, analogous to ER-associated protein degradation (ERAD; reviewed by Smith et al., 2012). The possibility of polyubiquitylation is supported by a survey of Arabidopsis ubiquitylome, in which ICL was identified as a ubiquitylated protein (Kim et al., 2013a). Furthermore, the PEROXIN4 (PEX4) gene, which may be involved in ubiquitylation, is necessary for the degradation of ICL and MLS (Zolman et al., 2005; Lingard et al., 2009). A third mechanism for peroxisomal degradation is pexophagy, a selective type of autophagy in which peroxisomes are targeted to the vacuole.

Pexophagy and its mechanism are well described in methylotrophic yeast and to a lesser extent in mammalian cells (reviewed by Till et al., 2012). Pexophagy typically removes obsolete or damaged peroxisomes. For example, peroxisomes are proliferated when methylotrophic yeast is grown in methanol, and excess peroxisomes are eliminated by pexophagy when methanol is replaced...
FIGURE 1 | A model of peroxisomal protein degradation in plant cells. (A) When autophagy occurs at a basal level or when a core ATG gene is missing, degradation of matrix proteins like ICL and MLS may depend on intraperoxisomal degradation (IPD) or peroxisome-associated protein degradation (PexAD). (B) Pexophagy may be induced by loss of LON2, inactivation of IPD and PexAD, or a developmental process in which peroxisomes are past a critical level of oxidative stress (for example, intensive fatty acid β-oxidation during early seedling growth). Under pexophagy-inducing conditions, phagophore initiates, expands, and forms the autophagosome to sequester and target the peroxisome to the vacuole for degradation. In this model, hydrogen peroxide (H₂O₂) is shown as a sole matrix-derived induction signal for pexophagy, although we cannot exclude the possibility of additional induction signals. Refer to the main text for possible contributions of PEX4, PEX14, NBR1, and ATG8 proteins to target recognition. Broken arrows indicate highly speculative steps.

DEGRADATION OF PEROXISOMES BY AUTOPHAGY IN ARABIDOPSIS

Early electron microscopy studies rarely include snapshots of autophagic degradation of peroxisomes in plant cells. However, there is a published example of autophagic vacuoles located near peroxisomes in castor bean endosperm, taken approximately 6 days after germination (Vigil, 1970). These snapshots alone were not sufficient for definitive evidence of plant pexophagy and required confirmation by immunoelectron microscopy and three-dimensional electron tomography.

A recent study employing a genetic suppressor screen provides evidence for pexophagy in plants. This was done by Dr. Bonnie Bartel’s group at Rice University in an attempt to identify the molecular function and targets of the LON2 protease (Farmer et al., 2013). The investigators screened for mutations suppressing lon2 phenotypes of defective β-oxidation activity and incomplete processing of peroxisome targeting signal (PTS) 2. In addition to the phenotypes, lon2 mutant cells had abnormally large spherical structures labeled by green fluorescent protein tagged with PTS (GFP-PTS), a widely used peroxisomal matrix marker. In contrast, the wild-type cells had small GFP-PTS puncta. Cloning of mutant genes that code for the lon2 suppressors resulted in the identification of several alleles of atg genes, specifically atg2, atg3, and atg7, two of which had been previously shown to cause defective autophagy (Doelling et al., 2002; Inoue et al., 2006). Double mutants of lon2 atg2, lon2 atg3, and lon2 atg7 all had normal β-oxidation and PTS2 processing, and had small GFP–PTS puncta. Moreover, endogenous ICL and MLS were stabilized in the double mutants, but not significantly in atg single-mutant seedlings. Farmer et al. (2013) presented a model in which autophagy removes a fraction of peroxisomes in wild-type Arabidopsis seedlings, while peroxisomal defects in lon2 mutation induce pexophagy (Figure 1B). Similar results were obtained by Dr. Mikio Nishimura’s group at National Institute for Basic Biology, Japan (Goto-Yamada et al., 2014). Lack of autophagy in the lon2 background appears to prevent the double-mutant seedlings from losing small peroxisomes, leading to the suppression of lon2 phenotypes (Bartel et al., 2014; Goto-Yamada et al., 2014). Although the precise function of LON2 in ICL and MLS degradation has yet to be defined, data described in Farmer et al. (2013), Goto-Yamada et al. (2014) indicate that LON2 protease plays a pivotal role in the IPD,
We can identify several questions concerning pexophagy in plant cells. First, why have there been few ultrastructure images suggestive of pexophagy in plant cells? The scarcity may be due to rapid targeting of autophagic vesicles to the vacuole (Zhuang et al., 2013). This possibility is supported by the observation of the phagophore-like structures in atg2, where autophagosomal formation may not be completed (Yoshimoto et al., 2014). In addition, the scarcity may result from a small developmental window in which seedling peroxisomes are rapidly transformed to leaf peroxisomes (Kim et al., 2013b). Hydrogen peroxide has been proposed as a signal for autophagy in plants (reviewed by Pérez-Pérez et al., 2012; Hackenberg et al., 2013). In support of this proposal, cat2 seedlings lacking a detectable level of catalase showed accelerated degradation of ICL and MLS (Lingard et al., 2009; see Figure 1B) compared to wild-type seedlings. Future work should clarify whether hydrogen peroxide acts as an upstream signal for both general and selective autophagy.

What proteins are necessary for recognizing peroxisomes targeted for autophagy? In methylotrophic yeast, a pexophagy receptor Atg30 bridges the molecular interaction between an autophagic complex and the peroxisomal proteins Pex3 and Pex14 (Farré et al., 2008; Zutphen et al., 2008). In mammalian cells, Pex14 interacts with LC3, an Atg8 homolog (Hara-Kuge and Fujiki, 2008). In addition, p62 and Neighbor of BRCA1 gene 1 (NBR1) may form a bridge between an ubiquitylated peroxisomal protein and LC3 (Kim et al., 2008; Deosarán et al., 2013). Intriguingly, an Arabidopsis ortholog of yeast Pex14 was identified from a genetic screen for mutants that showed stabilization of peroxisomal markers (Burkhart et al., 2013). It remains to be seen whether molecular interaction leading to pexophagy is conserved among distant eukaryotes (Figure 1).

ACKNOWLEDGMENTS
We apologize to colleagues whose work has not been mentioned because of space limitations. This work is supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT and Future Planning (2011-0010683) and by a grant from the Next-Generation BioGreen 21 Program (No. PJ009004), Rural Development Administration, South Korea.

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April 2014 | Volume 5 | Article 139 | 3
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