ARTICLE ADDENDUM

Plant Signaling & Behavior 10:11, e1101199; November 2015; Published with license by Taylor & Francis Group, LLC

From Arabidopsis to cereal crops: Conservation of chloroplast protein degradation by autophagy indicates its fundamental role in plant productivity

Masanori Izumi[1,2], Jun Hidema[2], and Hiroyuki Ishida[3,*]

[1]Frontier Research Institute for Interdisciplinary Sciences; Tohoku University; Sendai, Japan; [2]Department of Environmental Life Sciences; Graduate School of Life Sciences; Tohoku University; Sendai, Japan; [3]Department of Applied Plant Science; Graduate School of Agricultural Sciences; Tohoku University; Sendai, Japan

Autophagy is an evolutionarily conserved process leading to the degradation of intracellular components in eukaryotes, which is important for nutrient recycling especially in response to starvation conditions. Nutrient recycling is an essential process that underpins productivity in crop plants, such that remobilized nitrogen derived from older organs supports the formation of new organs or grain-filling within a plant. We extended our understanding of autophagy in a model plant, Arabidopsis thaliana, to an important cereal, rice (Oryza sativa). Through analysis of transgenic rice plants stably expressing fluorescent marker proteins for autophagy or chloroplast stroma, we revealed that chloroplast proteins are partially degraded in the vacuole via Rubisco-containing bodies (RCBs), a type of autophagosomes containing stroma. We further reported evidence that the RCB pathway functions during natural leaf senescence to facilitate subsequent nitrogen remobilization into newly expanding leaves. Thus, our recent studies establish the importance of autophagy in biomass production of cereals.

Keywords: autophagy, chloroplasts, crop plants, nitrogen remobilization, protein degradation, rice

*Correspondence to: Hiroyuki Ishida; Email: hiroyuki@biochem.tohoku.ac.jp

Submitted: 09/14/2015

Accepted: 09/24/2015

http://dx.doi.org/10.1080/15592324.2015.1101199

†Addendum to: Izumi M, Hidema J, Wada S, Kondo E, Kurusu T, Kuchitsu K, Makino A, Ishida H. Establishment of monitoring methods for autophagy in rice reveals autophagic recycling of chloroplasts and root plastids during energy limitation. Plant Physiol 2015; 167:1307:20:

Nutrients available for uptake by plant roots are often limited in nature, and accordingly plants recycle already assimilated nutrients to survive or to adapt their growth to environmental changes. Such nutrient recycling is an important determinant of productivity in crop plants even when they are fertilized in agricultural settings. For example, in rice (*Oryza sativa*), 45% of the total nitrogen in newly expanding leaves is remobilized nitrogen,[1] which is derived from the degradation of cellular components in older organs of the plant. The contribution of remobilized nitrogen to the grain-filling nitrogen can be as high as 50% to 90% in rice, wheat (*Triticum aestivum*), and maize (*Zea mays*),[2] which are the world's major cereals. As chloroplast proteins account for 75 – 80% of the total leaf nitrogen in C3 plants,[3] their degradation during leaf senescence is a critical initial process for subsequent nitrogen remobilization.

It was reported in early 1980s that the decrease of Rubisco protein, the most abundant stromal protein, is faster than that of overall chloroplast population during leaf senescence,[4] which indicates that Rubisco proteins are degraded either inside or outside of chloroplasts prior to the breakdown of entire chloroplasts. Indicative of a process involving Rubisco degradation after transport outside of chloroplasts, we discovered cytoplasm-localized small vesicles (0.5 – 1.0 μm diameter) containing Rubisco protein in wheat leaves, using immuno-electron microscopy.[5] We termed these Rubisco-containing bodies (RCBs). RCBs are more frequently observed in senescent leaves than in young leaves, and are surrounded by double membranes similar to those of autophagy-related structures called autophagosomes.[5] These observations led us to predict the existence of an autophagy process leading to senescence-induced degradation of chloroplast stroma.

Autophagy is a ubiquitous degradation system in eukaryotic cells.[6] During

autophagy, a portion of the cytoplasm including organelles is sequestered by an autophagosome and delivered to the vacuole in plants or lysosome in animals. The outer membrane of the autophagosome fuses with vacuolar/lysosomal membrane, thereby releasing the inner-membrane-bounded structure, termed the autophagic body, for degradation. Identification of autophagy-related *ATG* genes in budding yeast (*Saccharomyces cerevisiae*) shed light on the molecular basis of autophagy machinery. In land plants, advances in live cell-imaging techniques and reverse genetic approaches in the model plant *Arabidopsis thaliana* enabled *in vivo* detection of autophagy.[7] Numerous studies of Arabidopsis *atg* mutants revealed the conserved importance of plant autophagy in nutrient recycling, such as in the adaptive responses to starvation conditions,[8-10] similar to the case in yeast and animals. Our studies in Arabidopsis clearly demonstrated that the RCB pathway is a plant-specific autophagy process leading to piecemeal degradation of stromal proteins, and is responsible for nitrogen- or carbon-recycling during senescence or starvation.[11-15] These developments prompted us to validate the role of chloroplast autophagy in nutrient recycling of crop plants.

ATG genes are conserved in the rice genome,[16] and the retrotransposon *Tos17*–insertional knockout rice of OsATG7 (*Osatg7*) was previously isolated.[17] To clarify the progression of autophagy and the RCB pathway in rice plants, we generated transgenic rice stably expressing fluorescent marker proteins for autophagy or RCBs.[18] The vacuolar accumulation of autophagic bodies and RCBs were visualized with a monomeric red fluorescent protein-AUTOPHAGY8 fusion and a stroma-targeted green fluorescent protein (GFP), respectively, in roots and leaves. These phenomena were suppressed in the presence of an autophagy inhibitor, wortmannin, or in *Osatg7* mutants. GFP-labeled Rubisco holoenzyme enabled biochemical assays to investigate the activity of the RCB pathway, in which autophagy-dependent cleavage of free GFP from Rubisco-GFP is detected in response to its activation. This cleavage assay demonstrated that the RCB pathway is activated during starvation-induced leaf senescence. Thus, the establishment of fluorescent protein-based monitoring methods for autophagy in rice clearly indicates the contribution of autophagy to chloroplast protein degradation.[18]

In another approach, our physiological growth analysis of *Osatg7* plants demonstrated that deficiency in autophagy leads to low nitrogen use efficiency and reduced biomass production during vegetative growth.[19] In *Osatg7* leaves, the age-dependent decrease in soluble proteins including Rubisco was suppressed, and subsequent nitrogen remobilization into newly expanding leaves was partly compromised. Taken together, our 2 approaches establish that the autophagy-dependent RCB pathway is responsible for senescence-induced chloroplast degradation in rice plants, which facilitates efficient nitrogen remobilization for growth.[18,19]

A recent study revealed the central role of autophagy in nitrogen remobilization in maize,[20] and several reports have suggested that autophagy is important in responses to biotic or abiotic stresses in wheat.[21,22] Therefore, the manipulation of autophagy might be an effective strategy to improve the nitrogen use efficiency of important cereal crops or to enhance their tolerance of suboptimal agricultural conditions. However, many aspects of the molecular basis of plant autophagy remain unclear. Autophagy can be not only non-selective (starvation-induced), but also selective in terms of sequestering cargos.[6] In yeast, in contrast to the *ATG* genes related to autophagosomal membrane elongation such as *ATG5, ATG7* and *ATG9* that are required for autophagy, several *ATG* genes are only required for subcategories of autophagy. For example, *ATG17, ATG29* and *ATG31* are involved only in starvation-induced autophagy,[6] and the recently identified *ATG39* and *ATG40* are specifically required for selective turnover of the endoplasmic reticulum and the nucleus as receptor proteins for the cargo recognition.[23] Plant orthologs of such *ATG* genes have not been identified.[24] Because autophagy plays roles in wide-ranging processes, the manipulation of autophagy can lead to unexpected secondary effects. Identification of *ATG* genes specifically required for certain types of autophagy in Arabidopsis plants may provide appropriate avenues toward the manipulation of autophagy in crop plants.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This work was supported, in part, by KAKENHI (Grant Numbers 26506001 to M.I., 25119703, 15H04626 to H.I.), Building of Consortia for the Development of Human Resources in Science and Technology (to M.I.) and the Program for Creation of Interdisciplinary Research (to M.I.) in Frontier Research Institute for Interdisciplinary Sciences (Tohoku University).

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