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The autophagy gene, *ATG18a*, plays a negative role in powdery mildew resistance and mildew-induced cell death in Arabidopsis

Yiping Wang,[1,2] Yingying Wu[1,2] and Dingzhong Tang[1,*]

[1]State Key Laboratory of Plant Cell and Chromosome Engineering; Institute of Genetics and Developmental Biology; Chinese Academy of Sciences;

[2]Graduate University of Chinese Academy of Sciences; Beijing, China

Autophagy is a conserved intracellular recycling system that traffics cellular organelles and cytosolic proteins within lysosomes for reuse or breakdown in eukaryotes. Increased evidence indicates that autophagy is involved in programmed cell death and disease resistance in plants. We recently showed that *atg2*, *atg5*, *atg7* and *atg10* displayed early senescence and cell death in later growth stage under nutrient-rich conditions in *Arabidopsis thaliana*. These mutants also exhibited powdery mildew resistance and mildew-induced cell death. Salicylic acid (SA) signaling is required for *atg2*-mediated powdery mildew resistance, however, inactivation of SA signaling is not sufficient to fully suppress powdery mildew-induced cell death in *atg2* mutant.[1] Here, we show that *atg18a-2* is also resistant to the powdery mildew pathogen, *Golovinomyces cichoracearum,* and it shows mildew-induced cell death similar to the *atg2* mutant. Taken together, our study reveals that autophagy plays important roles in suppression of cell death and defense response to the biotrophic pathogen, the powdery mildew fungus. Future work on autophagy in plants will shine light on how autophagy is involved in cell death and defense response in plants.

Key words: autophagy, cell death, defense response, ATG2, ATG18a, powdery mildew

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*Correspondence to: Dingzhong Tang; Email: dztang@genetics.ac.cn

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Autophagy is a highly conserved biological process in eukaryotes from yeast to human. During autophagy, autophagosome (a double membrane structure) facilitates traffic of cellular organelles and cytosolic macromolecules into vacuole/lysosome for recycling or degradation.[2-5] A number of genes required for autophagosome formation were isolated by genetic studies in yeast.[5-7] Most of the core autophagy-related (*ATG*) genes are well conserved in higher eukaryotes, and to date, more than 30 Arabidopsis *ATG* genes have been identified.[8-10]

In plants, several *atg* mutants have been shown to display early senescence and/or spontaneous cell death in nutrient deficient or even in nutrient-rich conditions.[8,9,11-18] Recently, we showed that *atg2*, *atg5*, *atg7* and *atg10* exhibited early senescence and spontaneous cell death under nutrient rich conditions.[1] In addition, we showed that these mutants displayed enhanced disease resistance to the powdery mildew pathogen, *G. cichoracearum*. However, the *atg9* mutant was similar to wild type in the absence or presence of the powdery mildew pathogen, indicating that different *ATG* genes may have different or redundant function.

One interesting finding in our previous report was that the *atg2*-mediated resistance is dependent on SA signaling, however, inactivation of SA signaling can not fully suppress *atg2*-mediated powdery mildew-induced cell death, indicating that cell death could be uncoupled from resistance, and that cell death is not sufficient for powdery mildew resistance.[1]

Several studies show that autophagy contributes to pathogen-induced hypersensitive response (HR).[12,17,19,20] For instance, the restriction of HR cell death at the infection site was affected in *ATG6* silenced tobacco plants and *ATG6* antisense Arabidopsis plants.[12,19] To further study the role of autophagy in disease

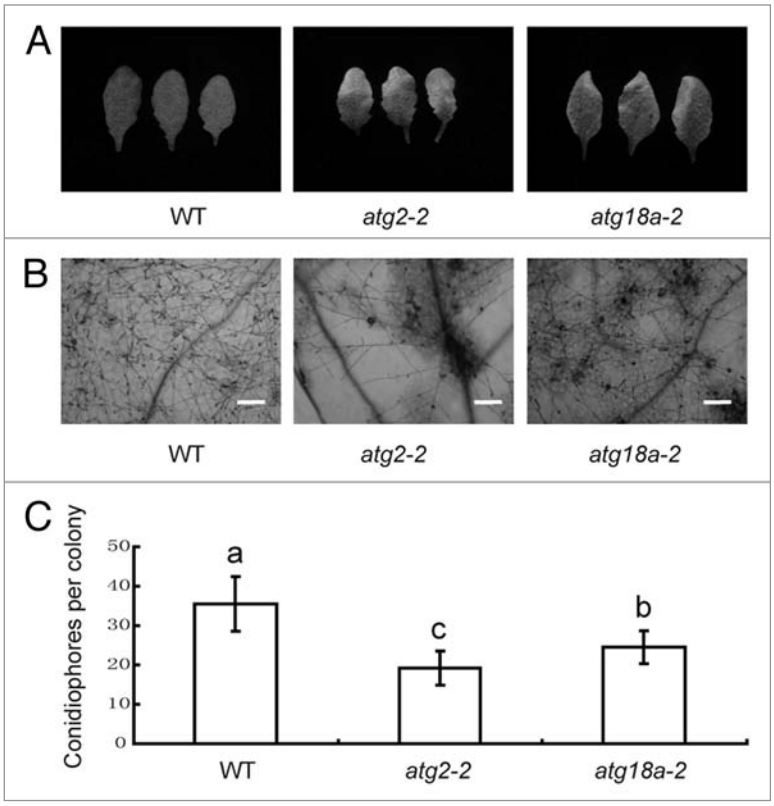

Figure 1. The *atg18a-2* mutant displays enhanced powdery mildew resistance and mildew-induced cell death. (A) Four-week-old wild type, *atg2*, *atg18a-2* plants were inoculated with *G. cichoracearum*. Leaves were removed from plants and photographed at 7 days post inoculation (dpi). (B) Trypan blue staining of leaves in (A). Bar = 100 μm. (C) Quantification of disease resistance by calculating the number of conidiophore per colony at 7 dpi in each genotype. Results represent mean ± SD in one experiment (n > 25). One-way ANOVA was performed for statistical analyses. Statistically significant differences between different genotype were indicated with different letters. Similar results were obtained from three independent experiments.

resistance, we examined the *atg18a-2* mutant for powdery mildew resistance. Previously, it has been shown mutation in *ATG18a* leads to early senescence phenotype.[14] To assess *atg18a-2* powdery mildew resistance, the *atg18a-2* mutant was grown under standard growth conditions for 4 weeks and the plant was infected with *G. cichoracearum*. As shown in **Figure 1A and B**, *atg18a-2* exhibited more pronounced cell death in the infected leaves than the wild type plant. To further investigate *atg18a-2* resistance phenotype, we monitored the fungal growth by counting the conidiophores in the infected leaves. The *atg18a-2* mutant supported significantly fewer conidiophores than wild type plants (**Fig. 1C**). These observations indicate that *ATG18a* also negatively affects both cell death and powdery mildew resistance, similar to *ATG2*, which was studied as well in **Figure 1**, as a positive control.

The interaction between autophage, cell death and disease resistance has emerged as an important topic in plant science. However, how autoghage affects cell death and resistance is not well understood. Hofius et al. reported that autophagy positively regulated disease resistance against virulent *Pto DC3000,* the Emwa isolate of *H. arabidopsidis* and avirulent bacteria,[20] while Lenz et al. showed that loss-of-function of *ATG5*, *ATG7*, *ATG10* and *ATG18a* conferred enhanced disease resistance to virulent bacteria *Pto DC3000*.[21,22] In another report, Yoshimoto et al. did not observe obvious difference in response to avirulent *Pto DC3000 avrRpm1*.[17] Similarly, we did not find significant differences between *atg2* and wild type upon infection with virulent or avirulent *Pto DC3000*. However, *atg2*, *atg5*, *atg7*, *atg10* and *atg18a-2* showed enhanced resistance to *G. cichoracearum*. Furthermore, enhanced disease resistance in *atg2* was correlated with increased accumulation of transcripts of defense-related genes, such as *PR1*.[1] Recently, Lenz et al. and Lai et al. reported that *atg5, atg7, atg10* and *atg18a* mutants exhibited enhanced susceptibility to *B. cinerea*,[21,23] indicating that autophagy positively affects defense response toward necrotrophic pathogen.

Accumulating evidence indicates that autophagy plays important role in plant defense response and disease resistance. However, there are still some debate about the role of autophagy towards pathogens. Here we showed *atg18a-2* also displayed enhanced disease resistance to powdery mildew, indicating that autophagy plays a negative role in disease resistance to biotrophic pathogens.

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