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SHORT TAKE

Autophagy mediates caloric restriction-induced lifespan extension in *Arabidopsis*

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Summary

Caloric restriction (CR) extends lifespan in various heterotrophic organisms ranging from yeasts to mammals, but whether a similar phenomenon occurs in plants remains unknown. Plants are autotrophs and use their photosynthetic machinery to convert light energy into the chemical energy of glucose and other organic compounds. As the rate of photosynthesis is proportional to the level of photosynthetically active radiation, the CR in plants can be modeled by lowering light intensity. Here, we report that low light intensity extends the lifespan in *Arabidopsis* through the mechanisms triggering autophagy, the major catabolic process that recycles damaged and potentially harmful cellular material. Knockout of autophagy-related genes results in the short lifespan and suppression of the lifespan-extending effect of the CR. Our data demonstrate that the autophagy-dependent mechanism of CR-induced lifespan extension is conserved between autotrophs and heterotrophs.

Key words: *Arabidopsis thaliana*; light intensity; caloric restriction; autophagy; longevity.

Arabidopsis is a monocarpic plant with a short life cycle that can be divided into four major stages: seedling development, vegetative development (or leaf rosette development), and partly overlapping flowering and rosette senescence (Boyes *et al.*, 2001). The lifespan of *Arabidopsis* extends from radicle emergence until complete senescence of the rosette and cessation of flowering. We found that decreasing the light intensity from 150 μE m^{-2} s^{-1} [standard conditions recommended for *Arabidopsis* (Hennig, 2010); hereafter referred to as normal light (NL)] to 100 μE m^{-2} s^{-1} (low light; LL) under constant photoperiod (16 h) delayed most of the developmental transitions in wild-type Columbia (WT) plants (Table S1) resulting in extension of the individual stages of life cycle (Fig. 1A, Table S2) and cumulative increase in the mean lifespan up to ~25% (Fig. 1A). To confirm that LL treatment mimics caloric restriction (CR), we measured photosynthetic capacity (SPAD values proportional to chlorophyll content) and glucose content in the rosette leaves at the onset of flowering. As expected, lowering light intensity was accompanied by decrease in both SPAD values and glucose content (Fig. S1).

It has recently been shown in yeast and animal models that CR promotes longevity via activation of autophagy (Madeo *et al.*, 2010; Rubinsztein *et al.*, 2011). To address possible roles of autophagy in LL-induced lifespan extension in *Arabidopsis*, we analyzed two autophagy markers, the degradation of the autophagic adapter protein neighbor of BRCA1 gene product (NBR1) by Western blots (Svenning *et al.*, 2011; Klionsky *et al.*, 2012) and the expression of *ATG8a* by real-time quantitative PCR (Thompson *et al.*, 2005) in the leaves of NL- and LL-grown WT plants at the onset of flowering and 10 days later. Under LL, degradation of NBR1 and upregulation of *ATG8a* over a 10-day period were, respectively, ~3.5 and ~2 times higher, as compared with NL-grown plants (Fig. 2), indicating that LL stimulates autophagy.

Next, we investigated whether autophagy is required for lifespan extension in *Arabidopsis* using knockout lines *atg5-1* (Thompson *et al.*, 2005) and *atg7-2* (Hofius *et al.*, 2009). It has previously been shown that autophagy-deficient mutants of *Arabidopsis* have enhanced expression of *ATG8* genes, especially under conditions inducing autophagy (Thompson *et al.*, 2005). Consistent with these data, knockout of either *ATG5* or *ATG7* led to upregulation of *ATG8a* in response to the LL conditions to the levels higher than in WT and complementation lines (Fig. 2B). At the same time, both *atg5* and *atg7* plants grown under LL revealed strong accumulation of NBR1, indicative of impaired autophagic flux (Fig. 2A). *ATG* knockout plants have previously been shown to exhibit an early-senescence phenotype even under favorable growth conditions (Liu & Bassham, 2012). Apart from the early onset and completion of rosette senescence, faster completion of flowering and accelerated silique shattering (Table S1), we found that the lengths of all major stages of the life cycle in *atg5* and *atg7* plants were reduced regardless of the light intensity (Fig. 1A, Table S2). As a result, the mean lifespan of *atg5* and *atg7* mutants was decreased by ~22% and ~12%, respectively, under NL, and by ~32% and ~22%, respectively, under LL. Collectively, these data demonstrate that autophagy attenuates transition through the successive stages of *Arabidopsis* life cycle and therefore is the prerequisite for longevity.

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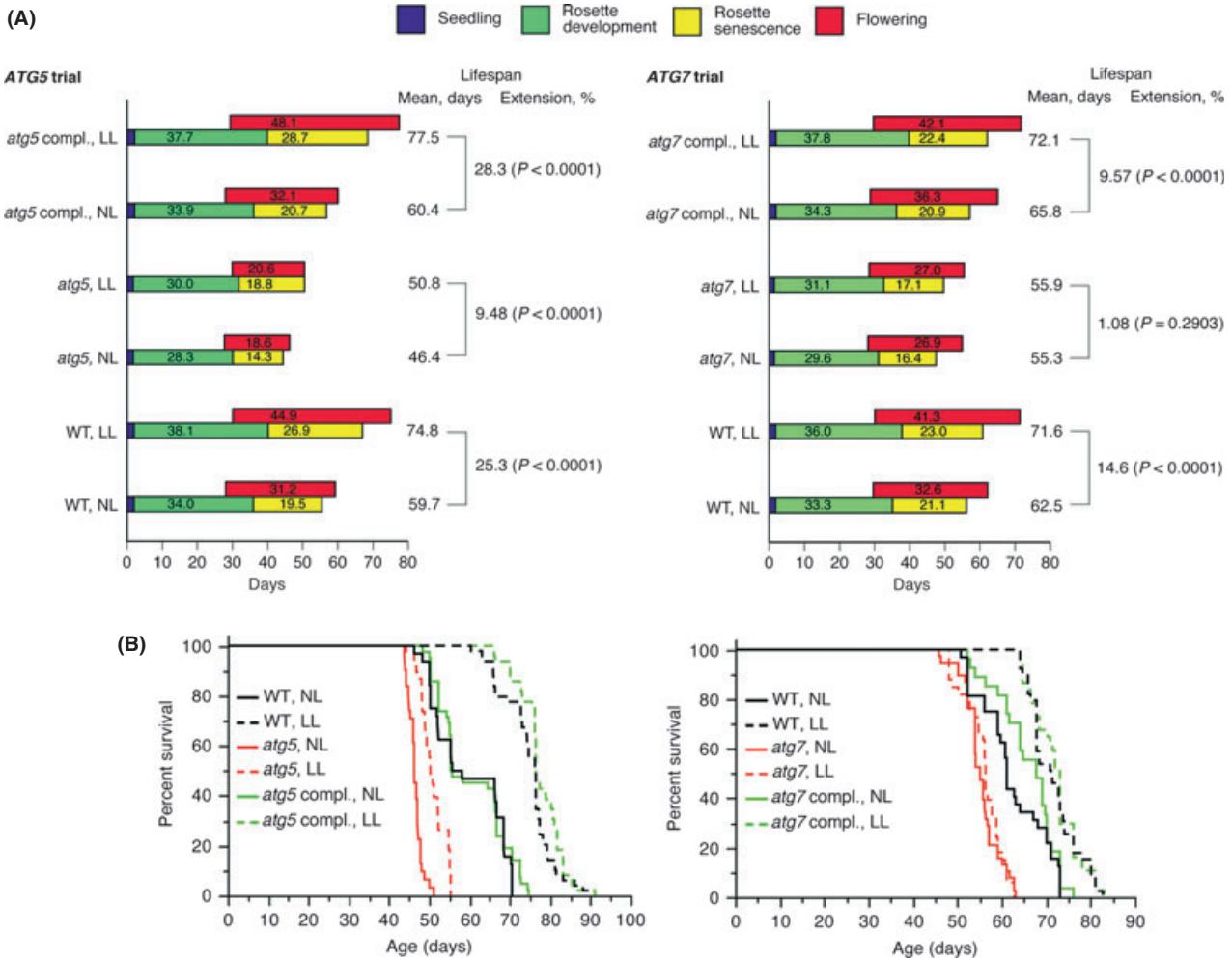

Fig. 1 The effect of light intensity and genetic inhibition of autophagy on *Arabidopsis* lifespan. Full legend is provided in supporting information online. (A) Life cycle of WT, *atg* and complementation lines grown under NL and LL. *X*-axis indicates days after radicle emergence. Numbers on the horizontal bars indicate mean length (days) of the developmental stage. Left and right graphs depict results of two representative independent trials, including *atg5* and *atg7* plants, respectively. *P* values indicate probability of lifespan extension by LL (Student's *t*-test). Detailed statistical analysis is provided in Tables S1 and S2. (B) Kaplan–Meier survival curves for the lifespan data from the same trials, as shown in A. *atg5* compl, complementation lines *atg5 pATG5::ATG5. atg7* compl, complementation lines *atg7 pATG7::ATG7.*

We have noticed that both *atg5* and *atg7* plants retained the ability to initiate CR-like response (i.e., decrease in both photosynthetic capacity and glucose content) to LL (Fig. S1). Therefore, we assessed whether these plants were able to sense CR and extend their lifespan similar to WT. The survival data show that the LL-induced increase in plant longevity was dramatically reduced (*atg5*) or completely abrogated (*atg7*) in the autophagic mutants, as compared with WT and complementation lines (Fig. 1). Thus, our findings reveal that intact autophagy machinery confers lifespan extension in *Arabidopsis* under the CR conditions.

Restricting uptake of calories has long been known as a simple and potent method for increasing longevity in heterotrophs (Mair & Dillin, 2008), and has recently been linked to the activation of autophagy (Madeo *et al.*, 2010; Rubinsztein *et al.*, 2011). Our work provides the first experimental setting for studying mechanisms increasing plant longevity under the CR and establishes autophagy as one of the crucial components. The plant autophagic machinery, and especially induction phase of plant autophagy, remains poorly understood, hampering our mechanistic insight into how autophagy is activated under the LL conditions. Once activated, autophagy can act in a number of pathways to confer cytoprotection (e.g., elimination of oxidized proteins and defective/old organelles) and sustain plant development (e.g., remobilization of nutrients) (Liu & Bassham, 2012), which collectively should lead to the lifespan extension.

Being an ephemeral monocarpic plant, *Arabidopsis* literally reproduces itself to death providing a tractable model to investigate lifespan regulation. Considering a central role of autophagy in the longevity of such evolutionary distant organisms as yeast, *Arabidopsis*, and mammals, autophagy is likely to play similarly important role in the longevity of polycarpic plants. Therefore, genetic or pharmacological manipulation of autophagy might open new venues for crop and tree production.

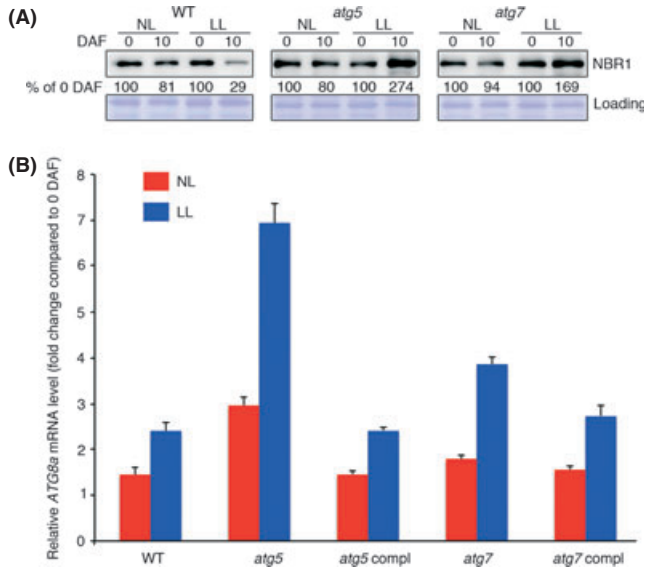

Fig. 2 Low light stimulates autophagy in *Arabidopsis*. Full legend is provided in supporting information online. (A) Western blot analysis of the total protein extracts from rosette leaves using anti-NBR1. The leaves were sampled at the onset of flowering (0 days after flowering, DAF) and after 10 days (10 DAF). Coomassie blue staining of the total protein (lower panel) was used as a loading control. Percentages shown below the upper panel correspond to the mean relative amount of NBR1 protein. Note dramatic decrease of NBR1 in LL-grown WT plants at 10 DAF, indicative of induction of autophagy. In contrast, *atg* plants accumulate NBR1 under corresponding conditions reflecting impaired autophagic flux. (B) Real-time quantitative PCR analysis of *ATG8a* expression in rosette leaves. The data represent mean relative level of *ATG8a* transcript at 10 DAF compared with 0 DAF. Error bars denote SD ($n = 3$). Low light induced significant upregulation of expression ($P < 0.0001$; Student's *t*-test) in all genotypes. *atg5* compl, complementation lines *atg5 pATG5::ATG5*. *atg7* compl, complementation lines *atg7 pATG7::ATG7*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Fig. S1 Low light induces caloric restriction in *Arabidopsis*

Data S1 Supporting Information containing full legends for Fig. 1 and Fig. 2, the legend for Fig. S1, experimental procedures with the list of primers and additional references.

Table S1 Timing of developmental transitions in wild-type, *atg* mutants and complementation lines grown under normal and low light conditions.

Table S2 Length of major stages of life cycle in wild-type, *atg* mutants and complementation lines grown under normal and low light conditions.