Research Subject : A03-2 様々な線質、線量率の宇宙放射線の急性影響
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この派遣による共同研究は、微小重力と高紫外線の複合環境ストレス下でのオートファジー誘導の分子機序を解明することを目的に、Prof. Salma Balazadeh と日出間との間で計画された。本プロジェクトを遂行するにあたり、本共同研究立案に至った研究に実質的に携わり、今後の若手研究者育成という視点から、当研究室の博士課程後期 3 年の Gonul Dundar が最適と考え、彼女を Prof. Salma Balazadeh Lab に派遣し、共同研究を実施するに至った。本プロジェクトの遂行にサポートをいただいた、新学術領域研究「宇宙に生きる」および国際活動支援班に深く感謝いたします。

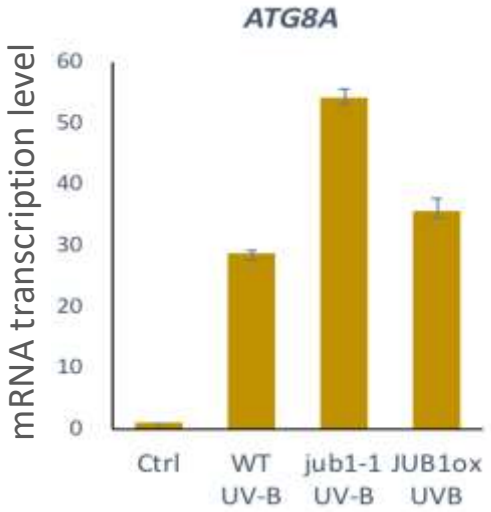
（日出間純）

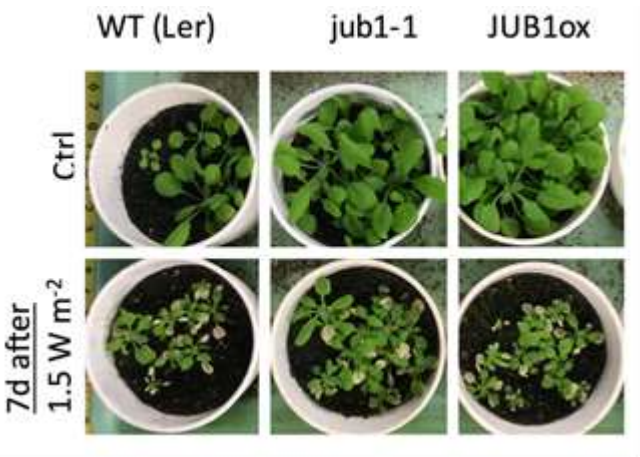
Autophagy, as one of the catabolic processes that is important for the survival of the cells by ensuring homeostasis in the cell under various conditions. Autophagy functions are known both in a bulk degradation process which cytoplasmic components degraded unselectively for nutrient recycling and also in a selective degradation for the elimination of damaged organelles by vacuolar hydrolyses enzymes.

In *Arabidopsis thaliana*, even though the core machinery of autophagy is well defined, its transcriptional regulation is largely unknown. The *Arabidopsis thaliana* NAC transcription factor JUNGBRUNNEN1 (AtJUB1), a growth regulator, showed higher expression after UVB treatment both in the photolyase and autophagy deficient plants. One of the groups in Max Planck Institute of Molecular Plant Physiology, Golm/Germany, Prof. Salma Balazadeh laboratory, studies the regulatory networks of transcription factors controlling the adaptation of plant growth to environmental stresses. In addition to JUB1 transcription factor (JUB1-TF), this group is also known that they identified other *Arabidopsis thaliana* NAC transcription factor, ATAF1, involvement in carbon starvation in *Arabidopsis* and contributed to the plant autophagy research field. In order to identify JUB1 TF role in the UVB induced-autophagy mechanism, therefore gaining a deeper understanding of the role of autophagy for UVB stress and microgravity responses, collaboration has been made with Prof. Balazadeh group.

Set of experiments are planned to show whether autophagy turnovers JUB1 or JUB1 as TF is involved in the induction of autophagy. Firstly, gene expression profilings accompanied with pull down assay or CHIP experiments are considered for analyzing which proteins or genes are interacted with JUB1. Live observation of the GFP fused JUB1 or PIF4 in the cells by fluorescence microscopy are done after UVB radiation. The autophagosome visualization by the fluorescent dye monodansylcadaverine (MDC) staining assay has been carried on in order to observe JUB1 and PIF4 proteins degradation by UVB induced autophagy.

Result of gene expression shows that autophagy marker gene *ATG8A* induction is more induced in JUB1 knock out lines (*jub1-1*-KO lines) after UV-B light, however the *ATG8* induction in JUB1 overexpressing (JUB1ox) plants was not different compared with that in wild type (WT) plants indicates that this autophagy activated in the downstream of the pathway (Fig. 1). Strong activation of autophagy might be caused by improved phenotype of the *jub1-1* KO line





which exhibited better growth than WT plants towards high UVB light (Fig. 2). Also, a photolyase (PHR1) mediated repair of UVB-induced DNA damage can be interpreted from the *JUB1* induction data. Both JUB1 and PHR1 shows interaction with PIF4 which is mediating skotomorphogenesis, and lack of this interaction in *jub1-1* KO plants suggests that it prevents repression of hypocotyl elongation after UV-B light treatment. Overall, with this collaboration in addition to gaining methodical skills to carry on experiments, it led us to involve in to enlighten the interesting piece of autophagy involvement in environmental stresses and TF contribution to the UVB response pathway which can be used as a target for the regulation of plant growth.

Figure 1. mRNA transcript levels of autophagy marker gene, *atg8a*, has been determined in wild-type (WT), jub1-1 KO (jub1-1) and JUB1-overexpressing (JUB1ox) trangenic Arabidopsis plants at 2 days after treatment with 1.5 W m^{-2} UVB light for 1 h.

Figure 2. UVB sensitivities of WT, jub1-1 and JUB1ox trangenic *Arabidopsis* plants. Plants were treated with 1.5 W m^{-2} UVB light for 1 h and pictures were captured at 7 days later.

As a last but not the least I would like to thank my supervisor Jun Hidema and host institute supervisor Salma Balazadeh for their fruitful support and suggestions. This collaboration project was supported by a MEXT Grant-in-Aid for Scientific Research on Innovative Areas, Japan "Living in Space," and International Activities Supporting Group (JP15H05945, JP15H05935, and JP15K21745).

(Gonul Dundar)