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Abstract of Doctoral Dissertation

Degree requested Doctor of Life Science Thais HUARANCCA REYES

Title of Doctoral Dissertation

Transcriptional activation and intracellular localization of *Arabidopsis* ubiquitin ligase ATL31 in defense and carbon/nitrogen response
(シロイヌナズナユビキチンリガーゼ ATL31 の遺伝子発現およびタンパク質細胞内局在制御を介した植物免疫と C/N 栄養環境適応機構の解析)

Plants are affected by abiotic and biotic stresses, with species preservation requiring them to perceive and develop optimal responses to environmental conditions. Transcriptional induction of stress-related genes and the appropriate intracellular delivering of proteins are required for plant adaptation. In this doctoral dissertation, I investigated the molecular mechanism of transcriptional activation and intracellular localization of an *Arabidopsis* ubiquitin ligase ATL31 which plays a critical role in plant defense responses to pathogen attack and carbon/nitrogen (C/N)-nutrient responses during post-germinative growth.

1. The *Arabidopsis* ubiquitin ligase *ATL31* is transcriptionally controlled by WRKY33 transcription factor in response to pathogen attack.

Transcriptional induction of stress-related genes involves the appropriate temporal and spatial binding of transcription factors to DNA sequences present in promoter regions of target genes. On the base of ATL31 research background, which *ATL31* gene expression is strongly induced in response to pathogen-associated molecular patterns (PAMPs) and pathogen infection, and which *ATL31* expression was found to highly correlate with the expression of the transcription factors *WRKY33* and *WRKY53*. Since WRKY33 has been reported to play essential role in plant defense, I further investigated detailed transcriptional regulation of *ATL31* by WRKY33 in response to pathogen attack. The results showed that WRKY33 positively regulates *ATL31* expression in *Arabidopsis* cells via specific W-box *cis*-acting element in the *ATL31* promoter. In addition, analyses of responses to bacterial and fungal PAMPs, flg22 and chitin, as well as to *Pseudomonas* bacteria in plants overexpressing *WRKY33* and those

with the *wrky33-1* mutant provided genetic evidence suggesting that the WRKY33 plays a positive role in plant disease resistance by promoting *ATL31* expression. Taken together, these findings indicated that WRKY33 acts as a transcription factor of *ATL31* and positively regulates its expression during activation of plant defense responses.

2. The ubiquitin ligase ATL31 regulates C/N-nutrient response through its association with a TGN-localized SNARE protein.

After the appropriate transcription and translation process, proteins are accurately sorted to a specific cellular localization through its intracellular trafficking between compartments. These trafficking pathways are involved in different cellular functions and responses to environmental stresses. The *trans*-Golgi network (TGN) is an important endomembrane organelle in plant cells where the endocytic and secretory pathways are merged. In this study, co-immunoprecipitation coupled to mass spectrometry analysis was established to identify the detailed function of ATL31 using *Arabidopsis* cultured cells expressing *ATL31*. The TGN-localized SNARE SYNTAXIN OF PLANTS 43 (SYP43) was identified as a novel ATL31 interacting protein. Co-immunoprecipitation and split-ubiquitin yeast two hybrid assays demonstrated that the ATL31 interacts with SYP43 *in vivo*. Moreover, microscope analysis showed that the ATL31 is localized on the TGN compartment and its localization is severely affected in *syp42 syp43* mutant. Interestingly, *syp42 syp43* mutant showed hypersensitive phenotype to C/N-nutrient stress condition similar to *atl31* mutant. These results suggested an important role of the TGN-localized SNARE in the ATL31 intracellular transport in response to abiotic stress.

Taken together, I concluded that WRKY33 transcription factor controls *ATL31* expression in plant defense response, and the intracellular trafficking of the ATL31 is associated with a TGN-localized SNARE protein in response to abiotic stress. This study revealed detailed molecular mechanism of transcriptional and post-translational regulation of the ubiquitin ligase ATL31 function, providing new insights of plant adaptation ability to multiple environmental stresses.