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Transcriptional activation and intracellular localization of Arabidopsis ubiquitin ligase ATL31 in defense and carbon/nitrogen response [an abstract of dissertation and a summary of dissertation review]

Author(s)

HUARANCCA REYES, Thais

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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, the author 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 basis 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, the author 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, the author 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.

In conclusion, the author has new findings on transcriptional activation and intracellular localization in plants, and these will contribute to promote advance in plant science and agriculture.

Therefore, we acknowledge that the author is qualified to be granted the Doctorate of Life Science from Hokkaido University.