APPLICATION NOTE

A1 R HD25, AX/AX R Confocal Microscope

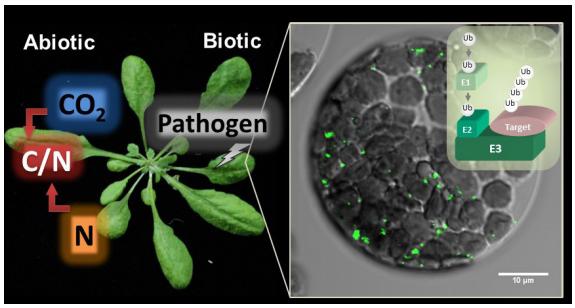
A confocal laser scanning microscope sheds light on the contribution of TGN/EE SNARE protein SYP61 and ubiquitin ligase ATL31 to C/N-nutrient stress tolerance in plants

Organisms can maintain homeostasis in response to environmental changes. Sugar (carbon (C) source) and nitrogen (N) are nutrients that play a key role in metabolism. In recent years, it has become increasingly clear that the relative availability of C and N (C/N-nutrient balance), in addition to the amount of C or N, affects the metabolism and the phase transition of plants. The amount of sugar supplied by photosynthesis, and nitrogen in the soil constantly fluctuate depending on daily and seasonal variations of sunlight conditions, temperature, rainfall, etc. In addition, the carbon dioxide concentration in the atmosphere keeps increasing. Since C/N imbalance causes early senescence and a decrease in biomass, it is important to understand the mechanism of plant adaptation to C/N-nutrient stress for sustainable crop production.

Dr. Takeo Sato, Dr. Junpei Takagi, and Dr. Yoko Hasegawa et al. of the Faculty of Science, Hokkaido University have been investigating intracellular signal transduction in plant C/N-nutrient responses, which affects defense against pathogens and plant development. Their previous studies using the model plant *Arabidopsis thaliana* have revealed that the membrane-localized ubiquitin ligase ATL31 regulates the C/N stress response through ubiquitination of 14-3-3 proteins. Although the transmembrane domains of ATL31 were shown to be important for its function, the intracellular localization and regulatory mechanism of ATL31 have not been clear.

This application note introduces a recently published report on plant C/N-nutrient stress adaptation, where a Nikon confocal microscope contributed to analyzing the regulation of ATL31 subcellular localization.

Keywords: confocal microscope, carbon/nitrogen-nutrient response, SNARE protein, ubiquitin ligase, *trans*-Golgi network (TGN), early endosome (EE), *Arabidopsis thaliana*



Ubiquitin ligase controls stress adaptation in plants.

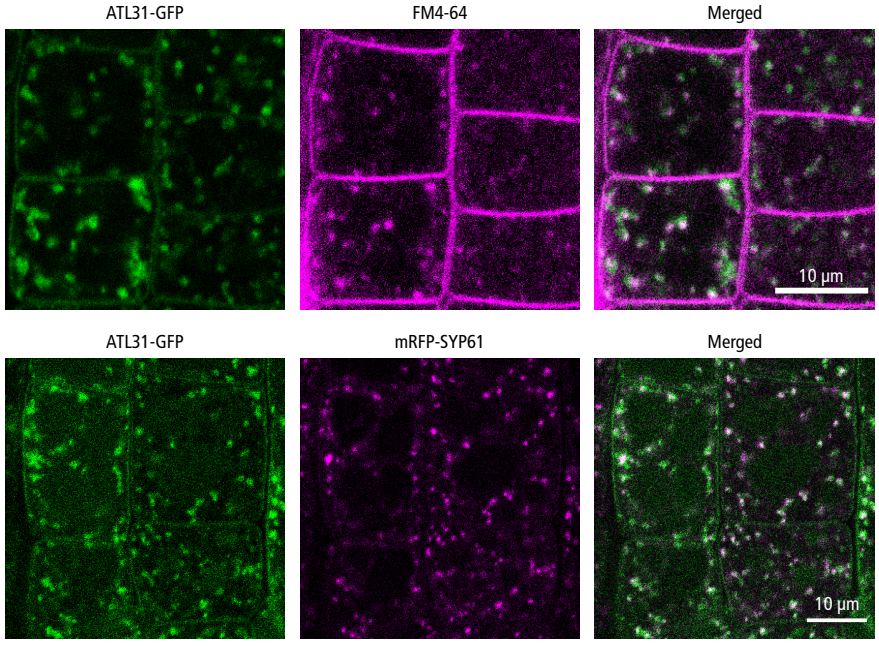


Figure 1. Confocal images of *Arabidopsis* root epidermal cells expressing ATL31-GFP

Top: Colocalization of ATL31-GFP with FM4-64
The root cells were stained with 2-µM FM4-64 for 2 minutes and observed 16 minutes later.
Bottom: Colocalization of ATL31-GFP with mRFP-SYP61

ATL31-GFP was colocalized with an endocytic tracer FM4-64 and the TGN/EE-localized SNARE protein SYP61 at the intracellular dot-like structures.

Microscope: A1 R HD25 confocal microscope
Objective: CFI Plan Apochromat Lambda 60X Oil (NA 1.40, WD 0.13)
Resolution: 1024×1024 pixels (cropped images)

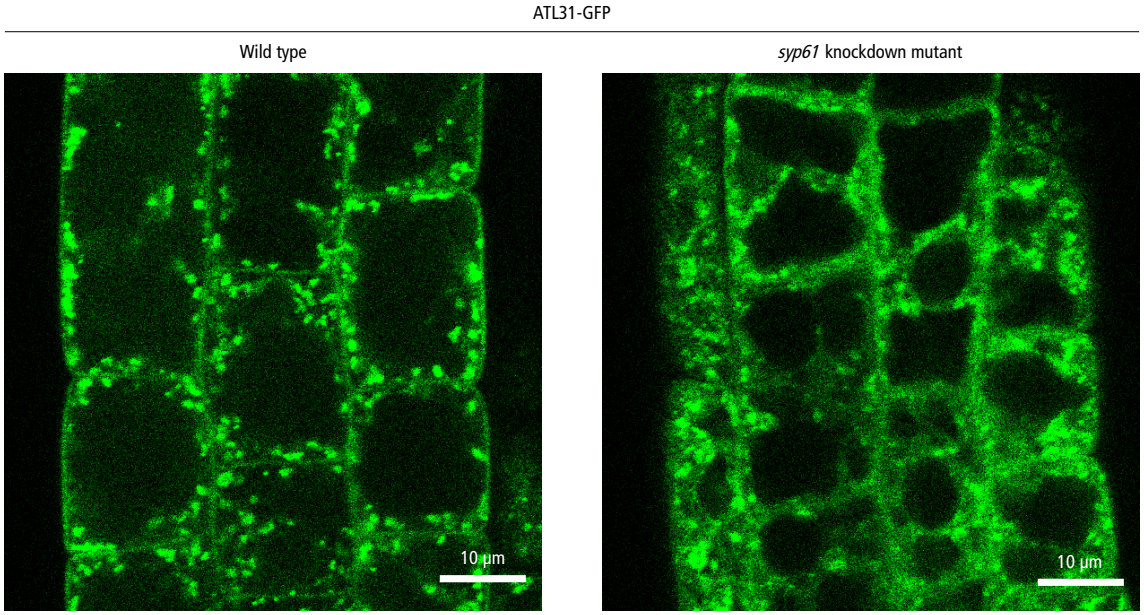


Figure 2. Confocal images of root epidermal cells of wild type and *syp61* knockdown mutant

Left: Wild type plant expressing ATL31-GFP
Right: *syp61* knockdown mutant expressing ATL31-GFP
Localization of ATL31-GFP was altered in the *syp61* knockdown mutant.

Microscope: A1 R HD25 confocal microscope
Objective: CFI Plan Apochromat Lambda 60X Oil (NA 1.40, WD 0.13)
Resolution: 1024×1024 pixels (cropped images)

Results and discussion

To clarify the subcellular localization of ATL31, *Arabidopsis thaliana* transformants expressing ATL31-GFP were observed with a confocal microscope. ATL31-GFP was found in the plasma membrane and the intracellular dot-like structures labeled with the endocytosis tracer FM4-64 (Fig. 1). This result suggested that ATL31 localizes at the *trans*-Golgi network (TGN), which functions as an early endosome (EE) in plants. Further colocalization analysis with the TGN-localized SNARE protein SYP61 confirmed that ATL31 localized at the TGN (Fig. 1).

In addition, biochemical analysis revealed that ATL31 interacts with SYP61. Since SNARE proteins are regulators of membrane trafficking that mediate the fusion of transport vesicles with target organelles, the intracellular localization of ATL31 in the *syp61* knockdown mutants was investigated. ATL31-GFP was abnormally dispersed in the cytosol in the mutant plants (Fig. 2). Moreover, physiological analysis showed that the *syp61* mutant was hypersensitive to C/N-nutrient stress. While the overexpression of ATL31 in wild-type plants conferred tolerance to C/N-nutrient stress as previously reported, it did not rescue the hypersensitive phenotype of the *syp61* mutant.

The researchers' results revealed that the TGN/EE-localized SNARE protein SYP61 contributes to C/N-nutrient stress tolerance in plants by regulating the proper localization of the E3 ubiquitin ligase ATL31. These findings could contribute to future plant breeding for sustainable and resilient crop production.

Confocal microscopes are powerful tools for imaging various cellular structures. In this example, the high-sensitivity GaAsP-PMT enabled the detection of very weak signals diffused in the cytosol of the mutant, with a high S/N ratio.

Reference

Hasegawa Y, Reyes TH, Uemura T, Baral A, Fujimaki A, Luo Y, Morita Y, Saeki Y, Maekawa S, Yasuda S, Mukuta K, Fukao Y, Tanaka K, Nakano A, Takagi J, Bhalerao RP, Yamaguchi J, Sato T (2022) The TGN/EE SNARE protein SYP61 and the ubiquitin ligase ATL31 cooperatively regulate plant responses to carbon/nitrogen conditions in Arabidopsis.
The Plant Cell, Volume 34, Issue 4, Pages 1354–1374
https://doi.org/10.1093/plcell/koac014

Product information

AX/AX R Confocal Microscope

Supports high-speed, high-resolution, large field-of-view confocal imaging, with reduced phototoxicity to living cells and photobleaching.

- High speed: Up to 720 fps (resonant at 2048 x 16 pixels)
- High resolution: Up to 8K (galvano)/ 2K (resonant)
- High throughput: Ultra-wide field of view of 25 mm

CFI Plan Apochromat Lambda D 60X Oil

This lens provides high image flatness, resulting in bright, clear images up to the edge of the large field of view of 25 mm. Chromatic aberration is corrected from 405 nm, enabling high quality multicolor imaging.

- NA: 1.42
- WD: 0.15
- PFS compatible