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Short Communication —Invited Paper—

Involvement of the membrane-localized ubiquitin ligase ATL8 in sugar starvation response in Arabidopsis

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Abstract As major components of the ubiquitin system, ubiquitin ligases mediate the transfer of ubiquitin to specific target substrates, thereby playing important roles in regulating a wide range of cellular processes. The Arabidopsis Tóxicos en Levadura (ATL) family is a group of plant-specific RING-type ubiquitin ligases with N-terminal transmembrane-like domains. To date, 91 ATL isoforms have been identified in the Arabidopsis genome, with some reported to regulate plant responses to environmental stresses. However, the functions of most ATLs remain unclear. This study showed that *ATL8* is a sugar starvation response gene and that *ATL8* expression was significantly increased by sugar starvation conditions but repressed by exogenous sugar supply. The ATL8 protein was found to possess ubiquitin ligase activity in vitro and to localize to membrane-bound compartments in plant cells. In addition, Starch Synthase 4 was identified as a putative interactor with ATL8, suggesting that ATL8 may be involved in modulating starch accumulation in response to sugar availability. These findings suggest that ATL8 functions as a membrane-localized ubiquitin ligase likely to be involved in the adaptation of Arabidopsis plants to sugar starvation stress.

Key words: SnRK1, starch synthase, sugar signaling, ubiquitination.

Ubiquitination is a post-translational modification, in which ubiquitin is covalently attached to the target proteins. Ubiquitination involves an enzymatic cascade, including the ubiquitin-activating enzyme E1, the ubiquitin-conjugating enzyme E2 and the ubiquitin ligase E3. Ubiquitination modulates many cellular processes, such as 26S proteasome-associated protein degradation, membrane trafficking and DNA-damage repair, playing a fundamental role in plant responses to abiotic and biotic environmental stresses (Hershko and Ciechanover 1998; Marino et al. 2012; Mukhopadhyay and Riezman 2007; Vierstra 2009). The *Arabidopsis thaliana* genome contains more than 1400 putative ubiquitin ligase genes, suggesting a vast diversity of specific target proteins. The Arabidopsis Tóxicos en Levadura (ATL) family is a sub-family of RING-type ubiquitin ligases specific to plant species, characterized by a Really Interesting New Gene (RING)-H2 variation of the RING-finger domain. To date 91 ATL members have been identified in Arabidopsis, 119 in rice and 82 in tomato (Lu et al. 2016; Serrano et al. 2006; Takai et al. 2002). Features common to all members of this family include one or two N-terminal transmembrane-like hydrophobic regions; a highly conserved 12–16 amino acid motif that often begins with glycine, leucine and aspartic acid residues designated the GLD motif; a RING-H2 type zinc finger domain; and a diverse C-terminal region thought to be involved in substrate recognition (Aguilar-Hernández et al. 2011; Guzmán 2014). ATLs have been shown to play several roles, including in responses to biotic and abiotic stresses. In Arabidopsis, ATL1, ATL2, ATL6, ATL9, ATL31 and ATL55/RING1 have been reported to function in plant immunity (Berrocal-Lobo et al. 2010; Lin et al. 2008; Maekawa et al. 2012; Reyes et al. 2015; Salinas-Mondragón et al. 1999; Serrano et al. 2014); and ATL78 was found to mediate ABA-dependent reactive oxygen species (ROS) signaling in response to drought stress as well as cold stress (Kim and Kim 2013; Suh et al. 2016). ATLs are also directly involved in plant nutrient availability. For example, ATL14, also called IDF1, was found to regulate iron uptake via the ubiquitin-dependent degradation of iron-regulated transporter1 (IRT1) proteins (Shin et al. 2013), and ATL80 was shown to be a negative regulator of phosphate mobilization (Suh and Kim 2015). We previously showed that ATL31/CNI1 (carbon/nitrogen insensitive 1) and its closest homologue ATL6 are required in the regulation of carbon/nitrogen responses during the post-germinative seedling growth transition, through the degradation of 14-3-3 proteins (Maekawa et al. 2012; Sato et al. 2009, 2011).

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However, the biochemical and physiological functions of many other members of the ATL family remain to be determined.

Apart from their essential role as primary metabolites, fueling plant growth and regulating metabolism, sugars act as messenger molecules in signal transduction (Rolland et al. 2006; Smeekens et al. 2010). Both sucrose and glucose can initiate changes in gene expression and have regulatory functions at many different levels. Hexokinase1 (HXK1) is a glucose sensor that regulates a broad spectrum of glucose responses and integrates the effects of glucose in signaling and metabolism (Moore et al. 2003). The sucrose non-fermenting1 (SNF1)-related protein kinase1 (SnRK1) is another important component involved in sugar signaling. SnRK1 functions as central energy regulator which is activated in response to energy deficit and maintain cellular homeostasis (Broeckx et al. 2016; Emanuelle et al. 2016). SnRK1 activity was recently reported to be directly modulated by binding to treharose-6 phosphate, suggesting that SnRK1 plays a sugar sensing role in Arabidopsis (Zhai et al. 2018). Global analysis methods, such as microarray and clustering, have begun to provide new insights into the identification of novel sugar-related components and their interaction with other signals, thereby revealing the molecular details of signaling pathways. To identify ATL genes particularly involved in sugar responses, a transcriptome database search of Arabidopsis was performed (Aoyama et al. 2017). It was found that a sugar-responsive, membrane-localized ubiquitin ligase, ATL15, may affect plant growth due to its possible role in extracellular sugar uptake (Aoyama et al. 2017). This study describes the identification of another ATL gene, *ATL8*.

The transcriptome profile of *ATL* genes involved in sugar responses was determined using Genevestigator (https://www.genevestigator.com), suggesting that *ATL8* (At1g76410) expression was increased under extended dark conditions but decreased by light treatment or exogenous sugar. Moreover, this gene was regarded as a marker of sugar-repressed genes although the function of ATL8 was totally unknown at that time (Graf et al. 2010). In this study we included a detailed expression analysis and biochemical characterization of ATL8. RT-PCR analysis was performed to determine *ATL8* expression levels under extended dark condition. *Arabidopsis thaliana* ecotype Columbia-0 (Col-0), regarded as WT, was grown for 3 weeks under short day (12 h light/12 h dark) conditions and subjected to extended darkness at the end of the night. *ATL8* expression level was significantly increased at 4 h and continuously expressed during the extended dark treatment (Figure 1A). To assess sugar-responsive *ATL8* expression, WT seedlings were grown on sugar-free 1/2 MS medium for 8 days and transferred to 1/2 MS medium containing 200 mM sucrose for 1 h, resulting in a dramatic reduction of *ATL8* expression level (Figure 1B, Supplementary Figure S1). These findings confirmed the results of transcriptome database analysis, that *ATL8* expression is regulated by extended darkness and by sugar application. To distinguish the light and sugar starvation signals under extended dark conditions, seedlings were exposed to the dark in the presence or absence of sugar. *ATL8* expression was not increased by extended dark treatment in the presence of sucrose, suggesting that cellular sugar availability, rather than light, is the primary signal that modulates *ATL8* expression level (Figure 1C). To determine its upstream regulatory pathway, *ATL8* expression was assessed in the *SnRK1α* knockdown mutant (*snrk1α1i/1α2*), an inducible RNAi knockdown

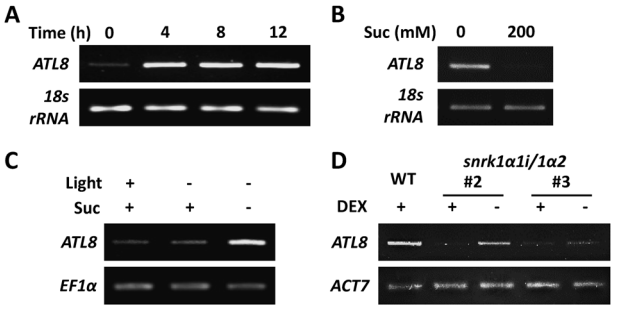

Figure 1. Expression of *ATL8* in response to light and sugar conditions. (A) *ATL8* expression was induced by extended dark treatment. RT-PCR analysis of *ATL8* mRNA transcripts in Arabidopsis WT plants were grown under SD (12 h light/12 h dark) conditions for 3 weeks, then subjected to extended darkness for the indicated times. Expression of *18s rRNA* was used as the internal control. (B) *ATL8* expression was reduced by exposure to sugar. WT seedlings were grown for 8 days in sugar-free MS medium and transferred to medium containing 0 or 200 mM sucrose for 1 h, and *ATL8* mRNA transcripts were determined by RT-PCR. *18s rRNA* was used as the internal control. (C) *ATL8* expression was induced by sugar starvation. WT seedlings were grown in MS liquid medium containing 1% sucrose for 7 days under continuous light, then transferred to dark conditions in the presence or absence of sugar for 2 days. Total mRNA transcripts of *ATL8* were analyzed by RT-PCR. *EF1α* was used as the internal control. (D) *ATL8* expression level in *snrk1α* mutants. WT and two lines (#2 and #3) of *snrk1α1i/1α2* seedlings (Sanagi et al. 2018) were grown for 7 days on sugar-free MS medium in the presence (+) or absence (−) of DEX, and *ATL8* mRNA levels analyzed by RT-PCR. *ACT7* was used as the internal control. Detailed methods are described in the Supplementary Methods.

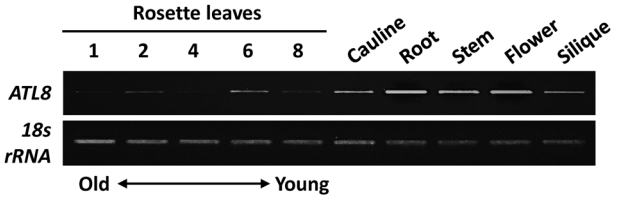

Figure 2. Expression patterns of *ATL8* in plant tissues. Semi-quantitative RT-PCR analysis of *ATL8* gene expression in tissues of 5-week-old WT Arabidopsis plants. 18s rRNA was used as the internal control. Detailed methods are described in the Supplementary Methods.

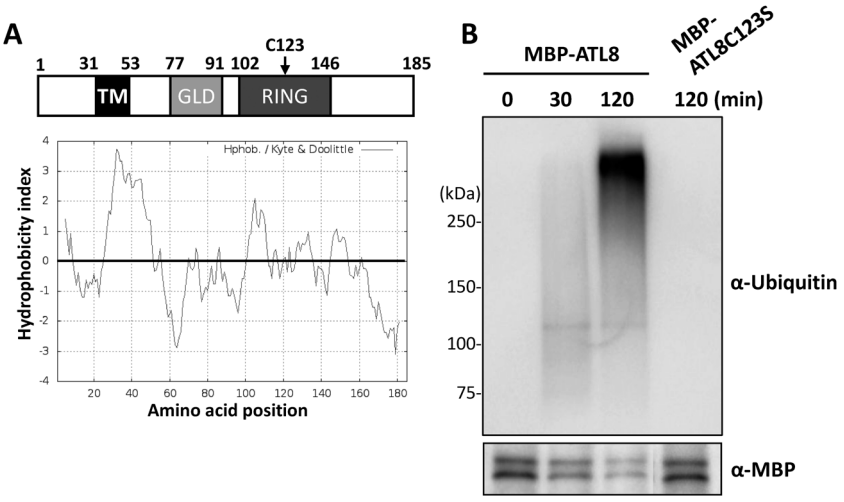

Figure 3. Schematic structure and in vitro ubiquitination assays of ATL8. (A) Schematic structure and hydropathy profile of ATL8 protein. Abbreviations: TM, transmembrane-like hydrophobic region; GLD, highly conserved region among ATLs including Gly-Leu-Asp residues; RING, RING-H2 zinc finger domain. The hydropathy profile was determined with ProScale software (http://web.expasy.org/protscale/). (B) In vitro ubiquitination assay of ATL8. Purified MBP-ATL8 protein was incubated with E1, E2, ubiquitin and ATP for 0, 30 or 120 min. RING-mutated ATL8 (MBP-ATL8C123S) protein was also incubated for 120 min. Ubiquitinated proteins were detected as a heterogeneous collection of higher molecular weight bands by western blotting with anti-ubiquitin antibody (upper panel). The presence of MBP-ATL8 protein was confirmed by western blotting with anti-MBP antibody (lower panel). Detailed methods are described in the Supplementary Methods.

mutant of *SnRK1α1* on the background of a *SnRK1α2* knockout mutant (Sanagi et al. 2018). RT-PCR analysis showed that *ATL8* expression was significantly reduced in the *snrk1α1i/1α2* seedlings, suggesting that sugar responsive *ATL8* expression is regulated by SnRK1 (Figure 1D). In addition, tissue-specific expression analysis indicated that *ATL8* is broadly expressed in all plant tissues, especially in the flowers, stems and roots (Figure 2).

ATL8 belongs to the group K ATL family, which includes *ATL80*, a gene involved in phosphate mobilization and cold stress response (Suh and Kim 2015). The ATL8 protein contains a single transmembrane-like hydrophobic amino acid region (31–53) at its N-terminus and a RING-H2 type zinc finger domain in its middle part (Figure 3A). To determine the biochemical properties of ATL8, its ubiquitin ligase activity was tested in vitro. Because the N-terminal hydrophobic region and the basic regions of ATLs inhibit sufficient protein expression in *E. coli*, these regions were therefore deleted, and residues 71 (valine)–185(phenylalanine) of ATL8 were fused to maltose binding protein (MBP) to generate the recombinant protein MBP-ATL8. The MBP-ATL8 protein was purified and subsequently incubated with E1, E2, ubiquitin and ATP for 0, 30 or 120 min, and the reactions were analyzed by western blotting with an anti-ubiquitin antibody. Ubiquitinated proteins appeared as a heterogeneous collection of higher molecular weight bands at 30 and 120 min (Figure 3B). A recombinant ATL8 protein containing a serine residue in place of a conserved cysteine residue in the RING domain (MBP-ATL8C123S) was also generated. The heterogeneous collection of proteins detected with the anti-ubiquitin antibody was completely abolished after incubation with MBP-ATL8C123S (Figure 3B), indicating that ATL8 has RING-type ubiquitin ligase activity in vitro. To investigate the subcellular localization of ATL8, since the ubiquitin ligase activity might lead to the instability of ATL8 protein as well as the associated target proteins, constructs encoding the ATL8C123S-GFP fusion protein and a membrane marker FLS2 receptor kinase fused with mCherry (FLS2-mCherry) were transiently co-expressed in *N. benthamiana* leaves. Confocal microscopy analysis showed that the ATL8C123S-GFP signal was present at the periphery of the cells, highly overlapping with FLS2-mCherry (Figure 4A). ATL8C123S-GFP was also localized to dot-like structures in the cytosol, suggesting that ATL8 also localizes to the endosomal compartment often observed in mesophyll protoplast cells (Supplementary Figure S2). Fractionation analysis was also performed by extracting crude protein lysate from the transgenic Arabidopsis seedlings continuously expressing ATL8C123S-GFP with or without detergent (1% Triton X-100), followed by ultracentrifugation to separate the soluble and insoluble fractions. ATL8C123S-GFP protein was enriched by immunoprecipitation using anti-GFP beads, followed by western blotting analysis. In the absence of Triton X-100, ATL8C123S-GFP was detected in the insoluble fraction but not in the soluble fraction (Figure 4B). In the presence of Triton X-100, however, ATL8C123S-GFP was detected in the soluble, but not the insoluble, fraction (Figure 4B). Taken together, these results indicated that ATL8 localizes to the

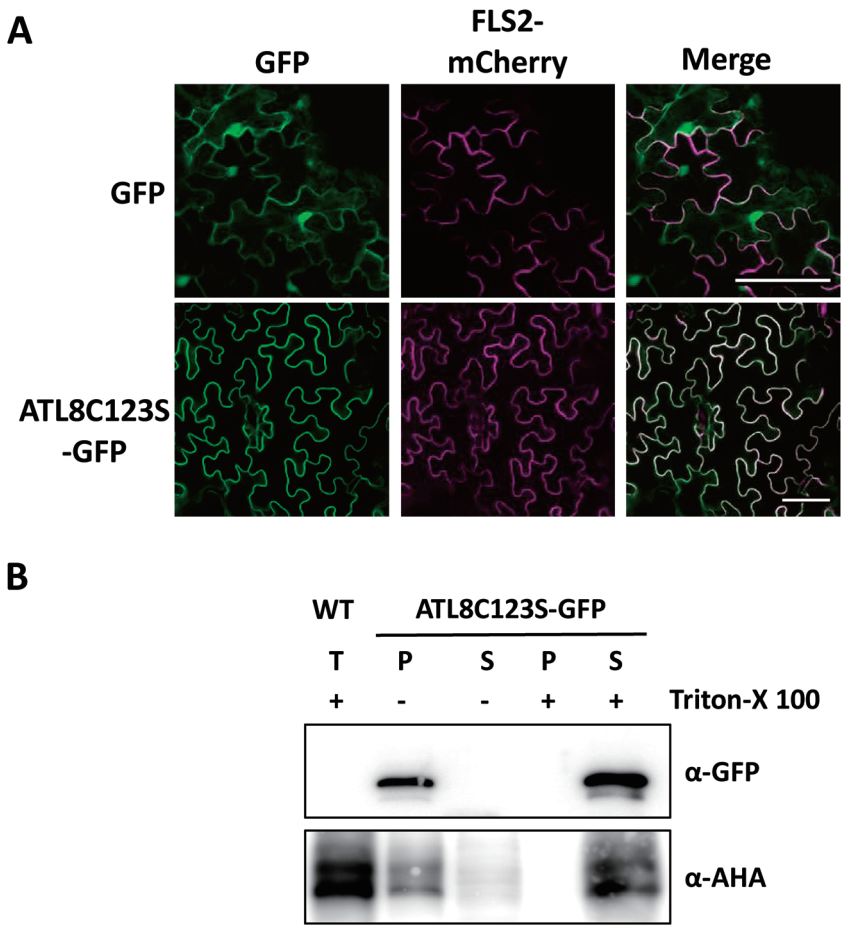

Figure 4. Subcellular localization of ATL8 protein. (A) Confocal laser microscopy showing the subcellular localization of ATL8. Fluorescence fusion protein ATL8C123S-GFP and the plasma membrane marker FLS2-mCherry were transiently co-expressed in *N. benthamiana* leaves. GFP was the control for fluorescent protein. Bars=100 μm. (B) Western blotting analysis using anti-GFP antibody of water-soluble (supernatant: S) and insoluble membrane (pellet: P) fractions of Arabidopsis transgenic plants expressing ATL8C123S-GFP fusion protein. H^+-ATPase (AHA) served as a marker for membrane-associated proteins. Extraction buffers contained no detergent (−) or 1% Triton X-100 (+). Total crude extract (T) from wild-type plants was the negative control for western blotting. Detailed methods are described in the Supplementary Methods.

Figure 5. Interaction of ATL8 with SS4 in split-ubiquitin yeast two-hybrid assays. Yeast cells were transformed with ATL8-Cub or ATL8C123S-Cub and Nub-SS4, -Nub. The transformants were streaked onto medium supplemented with X-gal. Blue patches indicate positive interactions. Abbreviations: Cub, the C-terminal half of ubiquitin; Nub, the N-terminal half of ubiquitin. Detailed methods are described in the Supplementary Methods.

membrane-bound compartments in plant cells.

To estimate the physiological function of ATL8 in plant cells, co-expressed genes were assessed by ATTED II database analysis (http://atted.jp/). *ATL8* expression was highly coordinated with the expression of genes involved in branched chain amino acid (BCAA) catabolism, including isovaleryl-CoA dehydrogenase (IVD); dihydrolipoamide branched chain acyltransferase (BCE2), also known as dark inducible3 (DIN3); branched chain alpha-keto acid dehydrogenase E1 beta (DIN4), branched-chain alpha-keto acid decarboxylase E1 Beta subunit (BCDH BETA1), and 3-methycrotonyl-CoA carboxylase (MCCB) (Supplementary Figure S3). These enzymes catalyze BCAA degradation, which is important in providing electrons transferred to the alternative respiratory chain through the ubiquinol pool via an ETF/ETFQO system during sugar starvation stress (Araújo et al. 2010; Schertl and Braun 2014). To further clarify the biochemical and physiological properties of ATL8, proteomics analysis was performed to identify

candidate ATL8 interactors. Wild-type and transgenic Arabidopsis plants continuously expressing ATL8C123S-GFP were immunoprecipitated with anti-GFP beads, followed by mass spectrometry analysis. This experiment identified Starch Synthase 4 (SS4) as a candidate ATL8 interactor. Starch is an insoluble glucan composed of two glucose polymers, amylopectin and amylose, as well as being the most widespread and abundant storage carbohydrate in plants. The regulation of starch amounts, synthesis and degradation is important in maintaining energy supply under dark and sugar starvation conditions (Usadel et al. 2008; Zeeman et al. 2010). The physical interaction between ATL8 and SS4 was assessed using a split-ubiquitin based yeast two-hybrid assay. Native ATL8 and RING-mutated ATL8C123S were fused to the C-terminal half of ubiquitin (ATL8-Cub and ATL8C123S-Cub) and SS4 was fused to the N-terminal half of ubiquitin (Nub-SS4), and both were used to transform yeast cells (Yasuda et al. 2017). Results showed that both native and mutated ATL8 interacted with SS4 (Figure 5). Unlike other starch synthase isoforms, which are responsible for either amylopectin or amylose elongation, SS4 plays a unique role in generating glucan primers to initiate starch granule synthesis. Chloroplasts of mutants lacking SS4 contain zero, one or rarely two large near-spherical starch granules, whereas chloroplasts of WT plants contain 5–7 granules with lenticular shape (Crumpton-Taylor et al. 2013; Lu et al. 2018; Seung and Smith 2019). Protein involved in starch initiation 1 (PII1) and protein targeting to starch 2 (PTST2) were recently identified as SS4 interactors, with both proteins playing important roles in regulating starch granule initiation by SS4 in Arabidopsis (Seung and Smith 2019; Seung et al. 2017; Vandromme et al. 2019). However, the mechanisms regulating SS4 function, such as transport to chloroplasts, catalytic activity, and protein degradation have not been determined. Ubiquitination could be a possible post-translational signal to modulate the SS4 function in plant cells. Further detailed biochemical and genetic analyses of the physiological function of ATL8 may provide new insights into ubiquitination-regulated starch synthesis and sugar starvation responses in plants.

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References

Aguilar-Hernández V, Aguilar-Henonin L, Guzmán P (2011) Diversity in the architecture of ATLs, a family of plant ubiquitin-ligases, leads to recognition and targeting of substrates in different cellular environments. *PLoS ONE* 6: e23934

Aoyama S, Terada S, Sanagi M, Hasegawa Y, Lu Y, Morita Y, Chiba Y, Sato T, Yamaguchi J (2017) Membrane-localized ubiquitin ligase ATL15 functions in sugar-responsive growth regulation in Arabidopsis. *Biochem Biophys Res Commun* 491: 33–39

Araújo WL, Ishizaki K, Nunes-Nesi A, Larson TR, Tohge T, Krahnert I, Witt S, Obata T, Schauer N, Graham IA, et al. (2010) Identification of the 2-hydroxyglutarate and isovaleryl-CoA dehydrogenases as alternative electron donors linking lysine catabolism to the electron transport chain of Arabidopsis mitochondria. *Plant Cell* 22: 1549–1563

Berrocal-Lobo M, Stone S, Yang X, Antico J, Callis J, Ramonell KM, Somerville S (2010) ATL9, a RING zinc finger protein with E3 ubiquitin ligase activity implicated in chitin- and NADPH oxidase-mediated defense responses. *PLoS ONE* 5: e14426

Broeckx T, Hulsmans S, Rolland F (2016) The plant energy sensor: Evolutionary conservation and divergence of SnRK1 structure, regulation, and function. *J Exp Bot* 67: 6215–6252

Crumpton-Taylor M, Pike M, Lu KJ, Hylton CM, Feil R, Eicke S, Lunn JE, Zeeman SC, Smith AM (2013) Starch synthase 4 is essential for coordination of starch granule formation with chloroplast division during Arabidopsis leaf expansion. *New Phytol* 200: 1064–1075

Emanuelle S, Doblin MS, Stapleton DI, Bacic A, Gooley PR (2016) Molecular insights into the enigmatic metabolic regulator, SnRK1. *Trends Plant Sci* 21: 341–353

Graf A, Schlereth A, Stitt M, Smith AM (2010) Circadian control of carbohydrate availability for growth in Arabidopsis plants at night. *Proc Natl Acad Sci USA* 107: 9458–9463

Guzmán P (2014) ATLs and BTLs, plant-specific and general eukaryotic structurally-related E3 ubiquitin ligases. *Plant Sci* 215–216: 69–75

Hershko A, Ciechanover A (1998) The ubiquitin system. *Annu Rev Biochem* 67: 425–479

Kim SJ, Kim WT (2013) Suppression of Arabidopsis RING E3 ubiquitin ligase AtATL78 increases tolerance to cold stress and decreases tolerance to drought stress. *FEBS Lett* 587: 2584–2590

Lin SS, Martin R, Mongrand S, Vandenabeele S, Chen KC, Jang IC, Chua NH (2008) RING1 E3 ligase localizes to plasma membrane lipid rafts to trigger FB1-induced programmed cell death in Arabidopsis. *Plant J* 56: 550–561

Lu KJ, Pfister B, Jenny C, Eicke S, Zeeman SC (2018) Distinct functions of STARCH SYNTHASE 4 domains in starch granule formation. *Plant Physiol* 176: 566–581

Lu Y, Yasuda S, Li X, Fukao Y, Tohge T, Fernie AR, Matsukura C, Ezura H, Sato T, Yamaguchi J (2016) Characterization of ubiquitin ligase SlATL31 and proteomic analysis of 14-3-3 targets in tomato fruit tissue (*Solanum lycopersicum L.*). *J Proteomics* 143: 254–264

Maekawa S, Sato T, Asada Y, Yasuda S, Yoshida M, Chiba Y, Yamaguchi J (2012) The Arabidopsis ubiquitin ligases ATL31 and

ATL6 control the defense response as well as the carbon/nitrogen response. *Plant Mol Biol* 79: 217–227

Marino D, Peeters N, Rivas S (2012) Ubiquitination during plant immune signaling. *Plant Physiol* 160: 15–27

Moore B, Zhou L, Rolland F, Hall Q, Cheng WY, Liu YX, Hwang I, Jones T, Sheen J (2003) Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. *Science* 300: 332–336

Mukhopadhyay D, Riezman H (2007) Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science* 315: 201–205

Reyes TH, Maekawa S, Sato T, Yamaguchi J (2015) The Arabidopsis ubiquitin ligase *ATL31* is transcriptionally controlled by *WRKY33* transcription fractor in response to pathogen attack. *Plant Biotechnol* 32: 11–19

Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: Conserved and novel mechanisms. *Annu Rev Plant Biol* 57: 675–709

Salinas-Mondragón RE, Garcidueñas-Piña C, Guzmán P (1999) Early elicitor induction in members of a novel multigene family coding for highly related RING-H2 proteins in *Arabidopsis thaliana. Plant Mol Biol* 40: 579–590

Sanagi M, Lu Y, Aoyama S, Morita Y, Mitsuda N, Ikeda M, Ohme-Takagi M, Sato T, Yamaguchi J (2018) Sugar-responsive transcription factor bZIP3 affects leaf shape in Arabidopsis plants. *Plant Biotechnol* 35: 167–170

Sato T, Maekawa S, Yasuda S, Domeki Y, Sueyoshi K, Fujiwara M, Fukao Y, Goto DB, Yamaguchi J (2011) Identification of 14-3-3 proteins as a target of ATL31 ubiquitin ligase, a regulator of the C/N response in Arabidopsis. *Plant J* 68: 137–146

Sato T, Maekawa S, Yasuda S, Sonoda Y, Katoh E, Ichikawa T, Nakazawa M, Seki M, Shinozaki K, Matsui M, et al. (2009) CNI1/ATL31, a RING-type ubiquitin ligase that functions in the carbon/nitrogen response for growth phase transition in Arabidopsis seedlings. *Plant J* 60: 852–864

Schertl P, Braun HP (2014) Respiratory electron transfer pathways in plant mitochondria. *Front Plant Sci* 5: 163

Serrano I, Gu Y, Qi D, Dubiella U, Innes RW (2014) The *Arabidopsis* EDR1 protein kinase negatively regulates the ATL1 E3 ubiquitin ligase to suppress cell death. *Plant Cell* 26: 4532–4546

Serrano M, Parra S, Alcaraz LD, Guzmán P (2006) The ATL gene family from *Arabidopsis thaliana* and *Oryza sativa* comprises a large number of putative ubiquitin ligases of the RING-H2 type. *J Mol Evol* 62: 434–445

Seung D, Boudet J, Monroe J, Schreier TB, David LC, Abt M, Lu KJ, Zanella M, Zeeman SC (2017) Homologs of PROTEIN TARGETING TO STARCH control starch granule initiation in Arabidopsis leaves. *Plant Cell* 29: 1657–1677

Seung D, Smith AM (2019) Starch granule initiation and morphogenesis-progress in Arabidopsis and cereals. *J Exp Bot* 70: 771–784

Shin LJ, Lo JC, Chen GH, Callis J, Fu H, Yeh KC (2013) IRT1 degradation factor1, a ring E3 ubiquitin ligase, regulates the degradation of iron-regulated transporter1 in Arabidopsis. *Plant Cell* 25: 3039–3051

Smeekens S, Ma J, Hanson J, Rolland F (2010) Sugar signals and molecular networks controlling plant growth. *Curr Opin Plant Biol* 13: 273–279

Suh JY, Kim SJ, Oh TR, Cho SK, Yang SW, Kim WT (2016) Arabidopsis Tóxicos en Levadura 78 (AtATL78) mediates ABA-dependent ROS signaling in response to drought stress. *Biochem Biophys Res Commun* 469: 8–14

Suh JY, Kim WT (2015) Arabidopsis RING E3 ubiquitin ligase AtATL80 is negatively involved in phosphate mobilization and cold stress response in sufficient phosphate growth conditions. *Biochem Biophys Res Commun* 463: 793–799

Takai R, Matsuda N, Nakano A, Hasegawa K, Akimoto C, Shibuya N, Minami E (2002) EL5, a rice *N*-acetylchitooligosaccharide elicitor-responsive RING-H2 finger protein, is a ubiquitin ligase which functions in vitro in co-operation with an elicitor-responsive ubiquitin-conjugating enzyme, OsUBC5b. *Plant J* 30: 447–455

Usadel B, Blasing OE, Gibon Y, Retzlaff K, Hohne M, Gunther M, Stitt M (2008) Global transcript levels respond to small changes of the carbon status during progressive exhaustion of carbohydrates in Arabidopsis rosettes. *Plant Physiol* 146: 1834–1861

Vandromme C, Spriet C, Dauvillée D, Courseaux A, Putaux JL, Wychowski A, Krzewinski F, Facon M, D'Hulst C, Wattebled F (2019) PII1: A protein involved in starch initiation that determines granule number and size in Arabidopsis chloroplast. *New Phytol* 221: 356–370

Vierstra RD (2009) The ubiquitin-26S proteasome system at the nexus of plant biology. *Nat Rev Mol Cell Biol* 10: 385–397

Yasuda S, Aoyama S, Hasegawa Y, Sato T, Yamaguchi J (2017) Arabidopsis CBL-interacting protein kinases regulate carbon/nitrogen-nutrient response by phosphorylating ubiquitin ligase ATL31. *Mol Plant* 10: 605–618

Zeeman SC, Kossmann J, Smith AM (2010) Starch: Its metabolism, evolution, and biotechnological modification in plants. *Annu Rev Plant Biol* 61: 209–234

Zhai Z, Keereetaweep J, Liu H, Feil R, Lunn JE, Shanklin J (2018) Trehalose 6-phosphate positively regulates fatty acid synthesis by stabilizing WRINKLED1. *Plant Cell* 30: 2616–2627