Ubiquitination in plant nutrient utilization

Gary Yates and Ari Sadanandom

School of Biological and Biomedical Sciences, Durham University, Durham, UK

INTRODUCTION

Our understanding of the layers of regulation that control the cell is deepening at a rapid rate. Much like how the discovery of microRNAs and epigenetics caused major rethinking of well-established gene control systems, protein modification processes are proving to have significant roles in the control of protein function. An example of this is ubiquitination, as a post-translational modifier it is well-known as a system involved in protein turnover, and to a lesser extent is known for its roles in membrane trafficking, DNA repair, chromatin remodeling, and hormone synthesis. However, recent publications have revealed that in addition to its extensive role in stress signaling, ubiquitination also has roles in nutrients utilization. We highlight the importance of the role ubiquitin (Ub) plays in plants ability to uptake and process nutrients using recent examples. It is clear that this area of research is in its infancy and much work has to be done in order to understand the extent to which ubiquitination influences this field.

UBIQUITINATION

Ubiquitin is a small seventy-six amino acid peptide that is highly conserved throughout eukaryotes. Ub is conjugated to the target protein through linkage between a C-terminal glycine and one or more of its seven possible lysine residues. Which of the seven lysine residue forms the attachment and the topology of the subsequent Ub chain directs the fate of the protein. Polyubiquitination via lysine 48 is usually associated with proteasomal degradation and in yeast and mammalian cells mono-ubiquitination and multiubiquitination are precursors to endocytic sorting and degradation via the lysosome and vacuole (Mukhopadhyay and Reizer, 2007). Once the target and the proteasome are connected, deubiquitinating enzymes remove the poly-Ub chain from the target protein, the Ub molecules are recycled, and the target is unfolded and fed into the 26S proteasome for proteolysis (Hartmann-Petersen et al., 2003). Opposed to lysine 48, attachment at lysine 63 is linked to endosomal degradation and trafficking (Duncan et al., 2006). Ub has five other lysines which can also take part in target conjugation, however, the precise physiological implications of these lysine linkages are yet to be discovered.

There are three main steps to Ub attachment to target proteins, each requiring a different enzyme type categorized as E1, E2, and E3. The first of these, E1, is an Ub activating enzyme. At a conserved cysteine residue, E1 forms a high-energy thioester bond with a C-terminal glycine of the Ub molecule, a step that requires ATP (Haftfeld et al., 1997). Ub, in its active form, is passed from the E1 to a cysteine residue in the E2 Ub-conjugation enzyme (E2C). which forms an intermediate complex. From here Ub is transferred to the lysine on the target protein, facilitated by the Ub ligase, E3. The E3 protein, of which there are two major subclasses in Arabidopsis, containing either a Really Interesting New Gene (RING)- or homologous to E6-AP carboxyl terminus (HECT)- domains, has the specificity to get the Ub to the appropriate target. This is achieved either by direct transfer from E2 to the substrate (RING domain E3) or by the formation of an Ub-E3 intermediate complex (HECT domain E3, Vierstra, 2009). In the Arabidopsis genome there are at least 16 genes encoding the Ub molecule itself, two genes for E1’s, at least 45 genes for E2’s, and over 1400

Keywords: ubiquitin, plants, nutrients, abiotic stress, signaling

Published: 12 November 2013
doi: 10.3389/fpls.2013.00452
Ubiquitin and nutrients

Arabidopsis

is found at six loci in the genome, all six transcripts are up-regulated significantly when Pi levels are low. The miR399 nucleotide sequence shows strong similarity to regions of the 5’ UTR of E2 ubiquitin-conjugation enzyme (UBC24), making the UBC24 transcript a likely target for decay by miR399 interaction (Allen et al., 2005). This is evident as the up-regulation of miR399 results in cleavage of the UBC24 transcript causing down regulation or even silencing of the UBC24 protein. When overexpressing miR399 in plants, accumulation of phosphate is observed and results in both increased uptake and increased translocation from the roots to the shoots (Chiou et al., 2006). Therefore given that there is interaction between miR399 and the UBC24 transcript, miR399 is most likely controlling phosphorus homeostasis by regulating both the uptake and trafficking of phosphorus, via control of the UBC24 protein level. The only suggested target of UBC24 is PHOSPHATE1 (PHO1), which is involved in the loading of phosphate to the xylem (Liu et al., 2012). Interestingly, the mammalian homolog of UBC24 is Apollon (Bartke et al., 2004) which acts as an inhibitor of cell death localized to the tGN and vesicles. Apollon has been shown to monoubiquitinate substrates in the presence of an E1 enzyme alone. Taken together, it is likely that the UBC24 also functions as an E2 with specific targets found in the tGN and vesicles.

Boron is another essential nutrient for plant life but is highly toxic in large quantities (Shorrocks, 1997). Plants depend on Borate to form a crosslinking dimer for proper formation of the cell wall component pectic polysaccharide rhamnogalacturonan II, without which plants do not develop normally and are largely non-viable (O’Neill et al., 2001). The uptake of boron depends on the transporter protein BORON TRANSPORTER1 (BOR1), and the boron acid channel protein NIP5;1. NIP5;1 is found on the soil facing surface of root cells and is essential for boron acid uptake in boron limited conditions (Takano et al., 2006). BOR1 is an efflux channel and is responsible for boron transport in low boron conditions (Takano et al., 2002, 2010). The BOR1 transporter utilizes the endocytic degradation pathway in high boron conditions where the excess is trafficked to the vacuole. This sorting of the loaded BOR1 transporter is made possible by its monoubiquitination or di-ubiquitination at lysine 590 and this is induced by high boron conditions. Mutation of BOR1 at lysine 590 abolishes both the ubiquitination and the degradation via the endocytic pathway (Takano et al., 2010). However, although ubiquitination is necessary for the sorting into multivesicular bodies, it is not required for BOR1 endocytosis, this process is very similar to the sorting of epidermal growth factor receptors (EGFR) in mammals (Huang et al., 2007). The E3 ligase responsible for targeting BOR1 is not yet known (Kanai et al., 2011). However, the EGFR undergoes ubiquitination by a WW (has two highly conserved tryptophans) containing domain E3 ligase which is homologous to HECT-type E3 ligase in Arabidopsis (Kraft et al., 2005).

There are similarities in the way BOR1 and IRT1 exploit the endocytic pathway for regulating their respective minerals, however, there is no current examples of plasma membrane transport proteins that involve processing by HECT-type E3 ligases (Mulet et al., 2013). However, it is probable, given the example of the Apollon, that there may be E2 enzymes involved in processing these membrane transporters without the need for a separate E3. Plants need nitrogen in high abundance, as it is a building block for proteins and nucleic acids. Nitrogen limiting conditions are widespread and as such plants have evolved a range of adaptive
responses to cope with the low availability. Amino acids are the major source of nitrogen transported in the xylem and phloem and they provide an important link between the nitrogen status of the roots and the rest of the plant (Paungfoo-Lonhienne et al., 2008). Amino acids can impede nitrogen uptake by roots and may play a major role in the regulation of transporters, providing part of a feedback system in sensing nitrogen (Miller et al., 2007). The import and export of amino acids in and out of the plant cells involve various membrane proteins and proton-gradient dependent transporters. The importing mechanisms are well-studied but little insight to the export processes exists (for review see Okamoto and Pilot, 2011). Despite the lack of understanding of the export mechanisms however, GLUTAMINE DUMPERRI (GUDI) overexpression in Arabidopsis leads to excess amino acid export. It is not understood exactly how this functions but mutant screens revealed it is the essential component for secretion of amino acids. Yeast-two-hybrid screens and glutathione S-transferase pull-down experiments show that GUDI interacts with a RING-type Ub E3 ligase; LOSSOFGUD2 (LOG2). LOG2 is required for the export of amino acids induced by GUDI (Pratelli et al., 2012). This interaction does not appear to be negatively regulating GUDI by Ub-based degradation but instead may serve as an activation step or perhaps GUD1 and LOG2 form part of a complex for another Ub target which could then act as a repressor or activator of that substrate (Léon and Haguenauer-Tsapis, 2009; Pratelli et al., 2012). Either way this work highlights a significant role for ubiquitination in the homeostasis of nitrogen, via amino acid regulation/secrection.

NITROGEN LIMITATION ADAPTATION (NLA) mutants in Arabidopsis are unable to respond to a transcriptional level to nutrient limiting conditions. NLA is the only identified component of the plants response to nitrogen limitation and is another RING-type E3 Ub ligase. Mutations within the RING motif of NLA are unable to initiate a response to low nitrogen conditions and they show the same phenotype as low nitrogen grown plants and induce early senescence. NLA is a positive regulator of the plants adaptability to nitrogen limiting conditions (Kant et al., 2011a). This identifies a major role for ubiquitination in the plants response to limited nitrogen and perhaps hints at a universal role for Ub in other areas of metabolism. This claim is reinforced by the fact that two E3 ligases have been shown to play a role in the carbon/nitrogen response (Sato et al., 2009). In addition to this, phosphate homeostasis is in part controlled by NLA in a nitrate dependent manner, and Pi shows an antagonistic cross-talk with nitrate in terms of both their accumulation and influence on the onset of flowering (Kant et al., 2011b). The PHOSPHATE TRANSPORTER1 (PHT1) group of proteins are crucial for Pi homeostasis and these are also regulated by NLA. PHOSPHATE2 (PHO2 also called UBC24), works in harmony with NLA at the plasma membrane, however, data suggest they act independently and both become targets of microRNAs (mirR827 and mirR399, respectively) under Pi deprivation (Huang et al., 2013). Interestingly PHO2 also induces PHT1 degradation under high Pi (Lin et al., 2013).

The Ub ligases ATL3 and ATL6 have been identified as crucial components of the proper recognition of the balance between carbon and nitrogen during germinative growth. The ratio of carbon to nitrogen is sensed and responded to by Arabidopsis and if the peripheral ratio is too high growth is arrested. The atl3 mutant is insensitive to carbon/nitrogen stress, while the overexpression of ATL3 resulted in the stress response but under normal conditions (Sato et al., 2009). ATL3 is therefore likely to negatively regulate proteins which detect the ratio of carbon to nitrogen under stress conditions. This underlines the importance ubiquitination has on the various stages of growth and development and again suggests that ubiquitination has a role in a plethora of functions in relation to nutrient homeostasis.

UBIQUITIN-LIKE PROTEINS AND NUTRIENT STARVATION

In addition to ubiquitination there are other small protein modifiers categorized as Ub-like proteins (Ubls) that can also be conjugated to target proteins. An example of this is the small Ub-related modifier (SUMO). The addition of SUMO to its target can have an effect on its targets ability to bind with the substrate. There are examples of SUMO preventing linkage between the target protein and its binding partner, and also examples of where the SUMOylation enables the binding of the target and its binding partner (for review see Geiss-Friedlander and Melchior, 2007; Miura and Haegewa, 2010). These mechanisms allow the process of SUMOylation to regulate some of the cells systems by acting as on/off switches for rapid responses to various changes in the cell and the environment. SUMOylation has also been linked to the area of nutrient regulation. Phosphate starvation dependent responses have been shown to be under the control of a SUMO E3 ligase; SIZ1 (Miura et al., 2005). SIZ1 controls the activation of the transcription factor PHOSPHA TE STARV A TION RESPONSE 1 (PHR1), which has been shown to bind to the promoter region of the majority of genes which are either up-regulated or down-regulated in response to phosphate starvation (Buasso et al., 2010). Mutant plants with altered SIZ1 show typical phosphate starvation responses when grown on normal conditions, this includes; discontinued primary root development, exaggerated lateral root growth, increased root hair development and excess anthocyanin accumulation, despite the internal phosphate levels being normal (Miura et al., 2005). Further to this debiquitination has also been shown to play part in the plants response to limited Pi. UBP14 is an Ub-specific protease that acts by modifying root morphology in Pi limited conditions (Li et al., 2010). Copper is needed by plants in small amounts but is essential for normal development. It is heavily involved in many aspects of growth and development including electron transport, redox reactions and as a cofactor for many metalloproteins. Copper is also toxic in surplus quantities, with excess build-up leading to chlorosis, root growth cessation, and even reduced iron uptake (Burkhead et al., 2009). In high copper conditions, SUMOylation is induced and SIZ1 mutant plants grow in excess copper conditions tend to show underdeveloped shoot growth compared to wild-type. SIZ1 mutants also accumulate more copper than any other metal relative to normal amounts. Further to this, there is a stark difference in the shoot-to-root ratio of copper in mutant plants compared to wild-type in high copper conditions and accumulation of SUMO1 conjugates is not observed in SIZ1 plants whereas it is stimulated by copper in the wild-type (Chen et al., 2011). SIZ1 also regulates nitrogen levels by its E3 SUMO ligase activity. A reduction of nitrate reductase activity is observed
in si2-2 plants. Data show this is due to SI2 targeting nitrate reductases (NIA1 and NIA2), which become highly active when SUMOylated (Park et al., 2011).

**TOOLS FOR TARGETING UBQUITIN AND UBQUITIN-LIKE PROTEINS AND COMPONENTS**

It is clear that target identification is key to revealing the influence of UbS in nutrient use efficiency in plants. More sensitive mass spectrometry methods are now available to identify targets for ubiquitination and SUMOylation in plants (Ellobeby and Coupland, 2010; Miller et al., 2010). Refined pull-down assays and in vitro ubiquitination assays now make finding targets of the process much more straightforward. For thioester bonds between either the E2 and a subunit of E3, or E3 and its substrate (Zhao et al., 2012). This allows for identifying the target protein and the residue to which the thioester bond forms enabling the isolation of forms of active signaling molecules. It can also be used to work back toward identification of the E3 and E2 if the target is already known.

**CONCLUSION**

From the evidence here, it is clear that ubiquitination and UbS play essential roles in nutrient uptake, trafficking and maintenance of many of the essential nutrients for plants. However, it is also clear that this area of research is ripe for new discoveries. It is highly likely that understanding the role of Ub in nutrient uptake and processing will provide much needed insight for the development of crops better suited to nutrient-deprived land.

**REFERENCES**

Allen, E., Xie, Z., Gustafson, A. M., and Carrington, J. C. (2005). *microRNA-directed phasing during trans-acting siRNA biogenesis in plants*. Cell 121, 207–221. doi: 10.1016/j.cell.2005.04.018

Berberon, M., Zelaya, E., Robert, S., Conesa, G., Carre, C., Pringle, J., et al. (2011). *Monoubiquitin-dependent endocytosis of the iron-regulated transporter t (IRT1) transporter controls iron uptake in plants*. Proc. Natl. Acad. Sci. U.S.A. 108, 2850–2855. doi: 10.1073/pnas.1011903108

Burkle, T., Pold, C., Chertkow, G., and Justus, S. (2004). *Dual role of BRUCE as an antiretroviral IAP and a chaperone E2/Ub ubiquitin ligase*. Mol. Cell 14, 801–811. doi: 10.1016/j.molcel.2004.05.018

Brian, J. E., and Lewin, B. M. (1999). *Plant responses to metal toxicity*. C. R. Acad. Sci. III 322, 43–54. doi:10.1074/jbc.R100000200

Burkhard, J. L., Reynolds, K. A., Abdul-Ghani, S. E., Cohu, C. M., and Pilon, D. (2010). *Regulation of phosphate homeostasis by microRNA in Arabidopsis*. Plant Physiol. 153, 1597–1611. doi: 10.1104/pp.110.159793

León, S., and Hagemann-Tripodi, B. (2009). *Ubiquitin ligase adaptors: regulators of ubiquitination and co-localization of plasma membrane proteins*. Exp. Cell Res. 315, 1574–1583. doi: 10.1016/j.yexcr.2008.11.014

Li, W.-C., Perry, P. I., Perdikis, N. N., and Schmidt, W. (2010). *Ubiquitin-Specific Protease 14 (UBP14) is involved in root responses to phosphate deficiency in Arabidopsis*. Mol. Plant 3, 212–223. doi:10.1093/mp/SSP096

Lin, W.-Y., Huang, K.-T., and Chou, T.-I. (2013). *NITROGEN LIMI-

TATION ADAPTION, a target of microRNA827, mediates degradation of plasma membrane-localized phosphate transporters to maintain phosphate homeostasis in Arabidopsis*. Plant Cell 25, 2368–2383. doi:10.1105/tpc.112.106656

Miller, A. J., Fan, X., Shen, Q., and Smith, S. J. (2007). *Amino acids and nitrate as signals for the regulation of nitrogen acquisition*. J. Exp. Bot. 58, 111–119. doi: 10.1038/sj.jxb.6201128

Miller, M. J., Barrett-Wilt, G. A., Hua, Z., and Vieira, R. D. (2010). *Proteomic analysis identify a diverse array of micro processes affected by small ubiquitin-like modifier conjugation in Arabidopsis*. Proc. Natl. Acad. Sci. U.S.A. 107, 16512–16517. doi: 10.1073/pnas.1005452107

Minnia, K., and Haagenaars, P. M. (2010). *Sumoylation and other ubiquitin-like post-translational modifications in plants*. Trends Cell Biol. 20, 220–225. doi: 10.1016/j.tcb.2010.01.007

Minnia, K., Lin, A., Sheldrick, A., Toh-e, S., Kardhiker, A. S., Krogan, N. J., et al. (2003). *The Arabidopsis SUMO E3 ligase SEZI controls phosphate deficiency responses*. Proc. Natl. Acad. Sci. U.S.A. 100, 7790–7795. doi: 10.1073/pnas.0505791102

Elenbasy, N., and Coupland, G. (2010). *Proteome-scale screens for small ubiquitin-like modifier (SUMO) substrates identify Arabidopsis proteins implicated in abscisic acid and auxin signaling*. Plant Cell 22, 1397–1409. doi: 10.1105/tpc.110.076452
Mukhopadhyay, D., and Riezman, H. (2007). Proteasome-independent functions of ubiquitin in endocytosis and signaling. Science 315, 201–205. doi: 10.1126/science.1137785

Muk, M. J., López-Torregrosa, V., Primo, C., and Manzanares, C., Tomish, L. (2013). Endocytic regulation of alkali metal transport proteins in mammals, yeast and plants. Curr. Genet. 59, 207–214. doi: 10.1007/s00294-013-0860-2

Ookuma, S., and Pilot, G. (2011). Amino acid export in plants: a missing link in nitrogen cycling. Adv. Plant Sci. 4, 453–463. doi: 10.1095/science.1105219

Park, B. S., Song, J. T., and Seo, H. S. (2011). Polar localization and degradation of Arabidopsis boron transporters through distinct trafficking pathways. Proc. Natl. Acad. Sci. U.S.A. 107, 5220–5225. doi: 10.1073/pnas.0910744107

Takano, J., Tanaka, M., Teyouda, A., Mino, K., Kasai, K., Fujii, K., et al. (2010). Polar localization and degradation of Arabidopsis boron transporters through distinct trafficking pathways. Proc. Natl. Acad. Sci. U.S.A. 107, 5220–5225. doi: 10.1073/pnas.0910744107

Mukhopadhyay, D., and Riezman, H. (2007). Proteasome-independent functions of ubiquitin in endocytosis and signaling. Science 315, 201–205. doi: 10.1126/science.1137785

Muk, M. J., López-Torregrosa, V., Primo, C., and Manzanares, C., Tomish, L. (2013). Endocytic regulation of alkali metal transport proteins in mammals, yeast and plants. Curr. Genet. 59, 207–214. doi: 10.1007/s00294-013-0860-2

Ookuma, S., and Pilot, G. (2011). Amino acid export in plants: a missing link in nitrogen cycling. Adv. Plant Sci. 4, 453–463. doi: 10.1095/science.1105219

Park, B. S., Song, J. T., and Seo, H. S. (2011). Arabidopsis nitrate reductase activity is stimulated by the E3 SUMO ligase AtSIZ1. Nat. Commun. 2, 481. doi: 10.1038/ncomms1408

Paungfoo-Lonhienne, C., Lonhienne, T. G. A., and Rentsch, D. (2008). Plants can use protein as a nitrogen source without assistance from other organisms. Proc. Natl. Acad. Sci. U.S.A. 105, 4724–4729. doi: 10.1073/pnas.0707120

Pratelli, R., Guerra, D. D., Yu, S., Wogulis, M., Kraft, E., Frommer, W. B., et al. (2012). The ubiquitin E3 ligase LOSS OF GDU2 is required for GLUTAMINE DUMPER1-induced amino acid secretion in Arabidopsis. Plant Physiol. 158, 1626–1642. doi: 10.1104/pp.111.191963

Sadanandom, A., Bailey, M., Furon, R., Lee, J., and Nishikawa, S. (2012). The ubiquitin-proteasome system: central modulator of plant signalling. New Phytol. 196, 15–28. doi: 10.1111/j.1469-8137.2012.03626

Saito, T., Maekawa, S., Yasuda, S., Sonoda, Y., Kato, E., Ichikawa, T., et al. (2009). Boron uptake and plant development under boron limitation. Plant Cell 21, 1498–1509. doi: 10.1105/tpc.106.041640

Shin, L., Lo, J., Chen, G., Callis, J., Fu, H., and Yeh, K. (2013). IRT1 DEGRADATION FACTOR1, a RING E3 ubiquitin ligase, regulates the degradation of IRON-REGULATED TRANSPORTER1 in Arabidopsis. Plant Cell 25, 1038–1051. doi: 10.1105/tpc.112.1115212

Shorrocks, V. M. (1997). The occurrence and correction of boron deficiency. Science 274, 846–849. doi: 10.1126/science.1062319

Ticconi, C. A., and Abel, S. (2004). Short on phosphate: plant surveillance and countermeasures. Trends Plant Sci. 9, 548–555. doi: 10.1016/j.tplants.2004.09.003

Vanderauwera, R. D. (2009). The ubiquitin-26S proteasome system at the nexus of plant biology. Nat. Rev. Mol. Cell Biol. 14, 1223–1233. doi: 10.1038/nrm2849

Zhao, Q., Liu, L., and Xie, Q. (2012). In vitro protein ubiquitination assay. Methods Mol. Biol. 876, 163–172. doi: 10.1007/978-1-61779-809-2_15

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 11 September 2013; paper pending published: 22 September 2013; accepted: 22 October 2013; published online: 12 November 2013.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

This article was submitted to Plant Nutrition, a section of the journal Frontiers in Plant Science.