RPN10: A Case Study for Ubiquitin Binding Proteins and More\textsuperscript{[OPEN]}

There is hardly any protein in the cell that does not encounter posttranslational modifications within its lifespan. Particularly pervasive is the covalent addition of ubiquitin and related polypeptides that are bound with high specificity to thousands of proteins, which then influence a large range of physiological and developmental processes in plants and other eukaryotes. Consequently, it is unsurprising that a web search for the term “ubiquitin” identifies several hundred articles in The Plant Cell, many of which have been highly cited.

Ubiquitin is typically attached via an isopeptide bond to accessible lysines through a reaction chain involving a ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzymes (E2), and finally ubiquitin ligases (E3). There are dozens of E2 enzymes and hundreds of E3 ligases in plants that act in pairs to ubiquitylate specific sets of substrates. In fact, mass spectrometric analyses of ubiquitylated proteins from Arabidopsis (Arabidopsis thaliana) have identified over a thousand substrates. Previously bound ubiquitins can also be targets, thus generating a wide array of chain topologies involving any of its seven lysines (Kwon and Ciechanover, 2017). How ubiquitin is added (single or multiple) and the architecture of the chain (linear, isopeptide, or branched) then determines the fate of the ubiquitylated protein through recognition by a collection of ubiquitin binding proteins with unique specificities. For example, ubiquitylated proteins destined for degradation by the 26S proteasome prefer substrates modified by isopeptide chains of ubiquitin internally linked through lysine-48.

The article that I highlight here concerns a pioneering discovery on one ubiquitin binding protein in Arabidopsis, called RPN10 (Smalle et al., 2003). In a search for ubiquitin receptors, Rick Vierstra and colleagues identified RPN10 by its ability to bind polyubiquitin chains in vitro (van Nocker et al., 1996). It then attracted interest for the realization that RPN10 was a conserved component of the 19S regulatory complex of the 26S proteasome, and this provided the first glimpse for how the 26S proteasome actually recognizes and captures its numerous polyubiquitylated substrates.

After this seminal finding, the Vierstra team moved on to study the function of RPN10 genetically. First, they studied moss using the recent advent of techniques for genetic ablation by homologous recombination (Girod et al., 1999), and then onto the flowering plant Arabidopsis using T-DNA-based insertion mutagenesis (Smalle et al., 2003), both published in The Plant Cell. The Physcomitrella patens rpn10 knockouts were viable but developmentally impaired in caulonema differentiation and unable to make gametophores (Girod et al., 1999). In Arabidopsis, disrupting RPN10 led to a pleiotropic phenotype at the level of plant development, sensitivity to phytohormones, and abiotic stress responses (Smalle et al., 2003). Importantly, rpn10 plants had increased levels of ubiquitylated proteins, supporting the notion that RPN10 helps to deliver a specific subset of short-lived targets to the 26S proteasome for breakdown. Mapping the ubiquitin binding site led to the identification of ubiquitin-interacting motifs (or UIMs) that are present in a wide array of binding proteins for ubiquitin-type polypeptides. One notable RPN10 substrate was the basic domain/Leu zipper transcription factor ABI5 central to abscisic acid (ABA) signaling, whose dramatically increased stability in rpn10 plants helped explain the ABA hypersensitivity of seedlings (Smalle et al., 2003). At that time, the authors could not imagine how many different ubiquitin E3 ligases actually impinge on ABA perception (Yu et al., 2015).

However, the story of RPN10 did not end there. One observation was that RPN10 was the only proteasome subunit that also existed in a free form untethered to the complex. The other was the curious unpublished result that 26S proteasome levels were elevated in the rpn10 background (Smalle et al., 2003). A decade later, this curiosity has been explained. From studies of 26S proteasome dynamics, the team discovered that proteasomes themselves are turned over by autophagy (Marshall et al., 2015). This degradation can be triggered by nitrogen starvation or inactivation of proteasomes either genetically or by inhibitors such as MG132. Surprisingly, inactivation leads to extensive ubiquitylation of the 26S complex followed by sequestration into the engulfing autophagic membranes. The team then learned that free RPN10 was the culprit. Through its UIM domain, RPN10 binds the attached ubiquitin moieties and then, at a slightly different UIM domain, also binds the ubiquitin-related polypeptide ATG8 that is at the center of the autophagic system (Marshall et al., 2015). ATG8’s job is to become tethered to the emerging autophagic membranes through lipidation and then to provide a docking platform for autophagic receptors recruiting appropriate cargo. By binding the ubiquitins on the 26S proteasome and ATG8, RPN10 provided the lynchpin for clearing defective proteasomes. This process was termed proteaphagy and was subsequently shown to be a conserved mechanism, albeit recruiting distinct receptors: RPN10 in plants (Marshall et al., 2015), Cue5 in yeast (Marshall et al., 2016), and p62 in mammals (Cohen-Kaplan et al., 2016). Further, this proteaphagy, along with RPN10, was also found to be important for several plant pathogens that exploit RPN10 to stimulate proteasome clearance as a mechanism to enhance bacterial proliferation and the promotion of virulence (Üstün et al., 2018).

But the story of RPN10 still does not end there. While all previously known autophagic receptors use a consensus ATG8-interacting motif to bind ATG8, the use of a UIM domain in RPN10 was unusual. Recently, through detailed mapping of the how RPN10 binds ATG8 followed by studies in Arabidopsis, yeast, and humans, Vierstra and colleagues discovered that the UIM in RPN10 is not novel but found in a wide variety of noncanonical autophagic adaptors and receptors, thus greatly...
expanding the mechanisms underpinning selective autophagy (Marshall et al., 2019). In fact, by studying mutant alleles of the critical disaggregate CDC48/p97 important for protein homeostasis, it was shown that a UIM-based autophagic route is critical for neuronal health in humans (Marshall et al., 2019).

In the end, this original work published in The Plant Cell (Smalle et al., 2003) on trying to understand how plant proteasomes recognize and degrade ubiquitylated proteins, led to a greater understanding of the reasons for the different chain topologies of ubiquitin, how cells control proteasome levels, how autophagy chooses appropriate cargo, and ultimately how various neurological diseases are linked to the UIM motifs first discovered in RPN10.

Pascal Genschik
Institut de Biologie Moleculaire des Plantes du CNRS
Strasbourg, 67084 cedex France
pascal.genschik@ibmp-cnrs.unistra.fr
ORCID ID: 0000-0002-4107-5071

REFERENCES


*Reference highlighted for the 30th anniversary of The Plant Cell.