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PROTEIN DEGRADATION

Catching ubiquitin

The function of the proteasome in ubiquitin-mediated protein degradation is well established, but little is known about how the proteasome recognizes its substrates. Now two studies have taken our understanding of this process one step further, by showing that the proteasome subunit Rpn13 is a high-affinity receptor for ubiquitin and recognizes its substrates through a new ubiquitin-binding domain.

> “Rpn13 is a high-affinity receptor for ubiquitin…”

Using a yeast two-hybrid assay, Husnjak *et al.* found that a conserved N-terminal region of human RPN13 interacts with ubiquitin. To test whether Rpn13 binds ubiquitin chains in the context of intact proteasomes, the authors first purified proteasomes from a yeast *rpn13*Δ mutant and assayed ubiquitin-chain binding.

They observed a reduction in ubiquitin-binding by *rpn13*Δ proteasomes, which is comparable to that observed in mutants of Rpn10, the only other proteasomal component known to function as ubiquitin receptor. Addition of recombinant Rpn13 to the *rpn13*Δ proteasome rescued the binding defect, thereby confirming that Rpn13 is a ubiquitin receptor. Analysis of the protein structure of yeast Rpn13 revealed that the N terminus of Rpn13, which binds to ubiquitin, has a configuration similar to the pleckstrin-homology domain (PHD). Therefore the authors named this domain pleckstrin-like receptor for ubiquitin (Pru). This is the first example of a PHD structure being found within the proteasome. In a separate study, Schreiner *et al.* found that murine Pru adopts a PHD fold structure and binds Lys48-linked diubiquitin with high affinity. In contrast to all other ubiquitin-binding proteins, RPN13 does not use α-helices to bind to ubiquitin, but instead uses the loop regions of the Pru. Mutants with amino-acid substitutions in the Pru loops did not bind to ubiquitin, which confirms that the loop regions of Pru are responsible for this interaction. Husnjak *et al.* determined the ubiquitin-binding residues of yeast Pru and showed that their mutation impairs binding.

To function as a proteasomal ubiquitin receptor, Rpn13 has to bind to ubiquitin and proteasome components simultaneously. So, is this the case? Schreiner *et al.* found that the proteasome subunit Rpn2 and the ubiquitin-binding surfaces of Rpn13 are largely independent; Rpn13 binds to Rpn2 through the Pru domain without disrupting the Rpn13 loops that bind to ubiquitin.

Husnjak *et al.* showed that Rpn13, like Rpn10, binds to ubiquitin-like (UBL)/ubiquitin-associated (UBA) proteins, which bind to and deliver ubiquitylated targets to the proteasome. Based on these findings, the authors propose that ubiquitin conjugates might bind to UBL/UBA proteins, which dock them to the proteasome and pass them to Rpn13 and Rpn10. Alternatively, UBL/UBA proteins and the intrinsic receptor might simultaneously bind to the targets.

Because the C terminus of Rpn13 binds to deubiquitylating enzymes, both studies propose that Rpn13 might couple the ubiquitin-chain recognition and disassembly at the proteasome, thereby linking two essential steps of selective protein degradation.

Francesca Cesari

ORIGINAL RESEARCH PAPERS Husnjak, K. *et al.* Proteasome subunit Rpn13 is a novel ubiquitin receptor. *Nature* **453**, 481–488 (2008) | Schreiner, P. *et al.* Ubiquitin docking at the proteasome through a novel pleckstrin-homology domain interaction. *Nature* **453**, 548–552 (2008)
FURTHER READING Hicke, L., Schubert, H. L. & Hill, C. P. Ubiquitin-binding domains. *Nature Rev. Mol. Cell Biol.* **6**, 610–621 (2005)