HIGHLIGHTS

PROTEIN DEGRADATION

The road to breakdown

Intracellular proteolysis in eukaryotes is mainly performed by the 26S proteasome, which is composed of the 19S regulatory complex bound to the proteolytic 20S core. Substrates destined for degradation are labelled with polyubiquitin (Ub_n), which is recognized by the 19S complex. Ub is released from substrates during protein degradation, but how it is removed, and the importance of this event, has remained unclear. Now, however, Deshaies and colleagues in *Science Express*, and Yao and Cohen in *Nature*, provide new insights.

Both groups began by studying the proteasome-mediated degradation of ubiquitylated substrates. When Deshaies and co-workers inhibited the proteolytic activity of the proteasome, they found that, although degradation of their chosen substrate was inhibited, it was still deubiquitylated in an ATP-dependent manner. Yao and Cohen used a Ub mutation that prevents it being released by deubiquitylating enzymes (DUBs), and compared the degradation of a Ub-mutant and wild-type fusion-protein substrate. They showed that the Ub-mutant substrate was degraded significantly slower than the wild-type substrate, which implies that deubiquitylation is a rate-limiting step for degradation.

Yao and Cohen showed that the intrinsic DUB in the 26S proteasome releases Ub_n from substrates by cleaving the Ub closest to the substrate. They also found that, although deubiquitylation *per se* is not ATP dependent, ATP is required for coupling to another substrate-processing step(s).

It was noted by both groups that a powerful DUB inhibitor did not affect this deubiquitylation, so what is the identity of the intrinsic DUB? Both groups eliminated known proteasome-associated DUBs, before looking at proteasome subunits. Of the non-ATPase 19S subunits in yeast, Yao and Cohen noted that Rpn11 (POH1 in humans) is the most conserved, which could indicate a catalytic function. Deshaies and colleagues also noted that Rpn11 contains a conserved $HXHX_{10}D$ sequence, which, in parallel work, was shown to underlie the ability of the proteasome-related COP9 signalosome to cleave a Ub-like molecule from its conjugates.

Because loss of intrinsic DUB activity is likely to hinder proteasome degradation and therefore be lethal, both groups tested the effect of mutating conserved Rpn11 residues on yeast growth. Overall, the two studies showed that, although the mutant proteasomes assembled normally, mutating the two histidines (H109 and/or H111) and the aspartate residue (D122) in the above motif resulted in lethality, and that mutating D142 affected growth. When Deshaies and co-workers mutated H109 and H111 to alanine, they showed that the mutant proteasomes were unable to deubiquitylate or degrade ubiquitylated substrates *in vitro*.

Rpn11/POH1 is therefore the intrinsic DUB in 26S proteasomes. Interestingly, both groups found that Rpn11/POH1 is not a cysteine protease like other DUBs, but is instead a Zn^{2+}-dependent protease — H109, H111 and D122 are putative Zn^{2+} ligands, and D142 presumably assists catalysis. These studies have identified both a new type of DUB that is essential for growth and a potential new family of Zn^{2+} metalloproteinases based on the $HXHX_{10}D$ sequence. They have also clarified the importance of deubiquitylation on the road to breakdown.

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References and links

ORIGINAL RESEARCH PAPERS Verma, R. *et al.* Role of Rpn11 metalloprotease in deubiquitination and degradation by the 26S proteasome. *Science Express* 2002 August 15 (DOI 10.1126/science.1075898) | Yao, T. & Cohen, R. E. A cryptic protease couples deubiquitination and degradation by the proteasome. *Nature* 2002 September 1 (DOI 10.1038/nature01071)