A set of surgical chain saws

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The EMBO Journal (2009) 28, 615–616. doi:10.1038/emboj.2009.52

A biochemical tour de force in this issue of The EMBO Journal suggests that JAMM/MPN⁺-domain containing deubiquitinating enzymes specifically disassemble K63-linked ubiquitin chains.

A constantly changing environment requires cells to orchestrate the activities of signal transduction pathways with high temporal and spatial precision. A powerful tool to regulate signalling is the reversible modification of proteins with ubiquitin chains, as it can alter the localization, activity or stability of modified substrates (Kerscher *et al.*, 2006). Cells have at their disposal hundreds of enzymes to assemble ubiquitin chains, and in many cases these ubiquitination enzymes are crucial components of signalling pathways.

The assembly of ubiquitin chains depends on the formation of an isopeptide bond between the carboxy-terminus of ubiquitin and a lysine in a substrate-attached ubiquitin. Depending on which of the seven lysine residues in ubiquitin is used for chain assembly, chains differ in structure and function. Although chains linked through K11 or K48 of ubiquitin send proteins for degradation by the 26S proteasome, those connected through K63 perform mostly nonproteolytic tasks (Kerscher *et al.*, 2006; Jin *et al.*, 2008). K63-linked chains have important functions in activating kinases in the NF- κ B pathway, orchestrating events during DNA repair or changing the localization of membrane proteins.

The dynamic nature of signalling often demands that ubiquitin chains are rapidly disassembled. This is the task of ~100 human deubiquitinating enzymes (DUBs), most of which are cysteine proteases (Nijman *et al.*, 2005). Only few DUBs, such as proteasomal Poh1, lack active-site cysteines, and instead contain a JAMM/MPN⁺-domain (Yao and Cohen, 2002; Ambroggio *et al.*, 2004). These JAMM-DUBs are metalloproteases, which require a Zn²⁺ ion coordinated in their active site to cleave the ubiquitin isopeptide bond. The specificity of JAMM-DUBs toward different ubiquitin chains has not been well understood.

Originally planning to purify proteins binding to ubiquitin chains, Cooper *et al.* incubated ubiquitin chains of specific linkage with extracts of human cells (Cooper *et al.*, 2009). To inhibit the abundant DUBs, they treated the extracts with NEM, which alkylates active-site cysteine residues. As expected, NEM ablated the capability of extracts to disassemble K48-linked chains, indicating that the bulk of K48-specific DUB activity is provided by cysteine-protease DUBs. By contrast, the full stabilization of K63-linked chains in extracts required the chelation of Zn²⁺, which suggested that a JAMM-DUB specifically disassembles K63-linked ubiquitin chains, prompting the authors to isolate this selective ‘chain saw’ using biochemical fractionation.

Although DUBs are notoriously ill-behaved *in vitro*, the authors succeeded in following the enzymatic activity over seven purifica-

tion steps, until they narrowed it down to an ~600 kDa complex. Protein identification by mass spectrometry revealed that the apparent 600 kDa complex was in fact a mixture of three complexes, all of which contained JAMM-domain proteins: the 19S proteasomal cap, including the JAMM-DUB Poh1; the CSN/signalosome with the Nedd8-specific catalytic subunit Csn5; and a novel complex, termed BRISC, which included the JAMM-DUB Brcc36. Subsequent experiments showed that Poh1 and BRISC exclusively disassemble K63-linked ubiquitin chains, whereas chains of other linkages are left untouched. Interestingly, another JAMM-DUB, AMSH, also disassembles only K63-linked ubiquitin chains, suggesting that the specificity for K63-linked chains may be a common property of JAMM-DUBs (Sato *et al.*, 2008).

The apparent K63-specific DUB activity of the CSN, however, turned out to be entirely due to co-purifying BRISC. Indeed, Cooper *et al.* (2009) show a tight interaction between the CSN and BRISC, thereby linking deneddylation (by Csn5) and deubiquitination (by Brcc36). By regulating the neddylation status of cullin proteins, the CSN controls the activity of cullin-RING ubiquitin ligases, some of which modify substrates in response to DNA damage (Petroski and Deshaies, 2005). The repair of DNA damage also relies on the decoration of histones with K63-linked chains, and a Brcc36-complex slightly different from the BRISC is targeted to sites of DNA damage (Sobhian *et al.*, 2007). The interaction between the CSN and the BRISC underscores the tight coupling of multiple ubiquitination and deubiquitination events during signalling.

This study raises the interesting question of why the proteasome harbours a K63-specific DUB, although most of its substrates are decorated with K48- or K11-linked chains. Poh1 was earlier identified as a proteasomal DUB removing the complete ubiquitin chain from a substrate before substrate degradation in the proteasomal chamber (Yao and Cohen, 2002). This activity of Poh1 is dependent on ATP-hydrolysis, and thus, presumably, on unfolding of substrates by the AAA-ATPases of the proteasomal cap. By contrast, the ATP-independent activity of Poh1 described here could allow the proteasome to spare proteins modified with K63-linked chains from degradation. Poh1 could also oppose ubiquitination enzymes such as Hul5, which extend K63-linked chains on proteins already bound to the proteasome to inhibit their premature dissociation (Crosas *et al.*, 2006). Finally, K63-linked chains might in some instances promote proteasomal degradation, as suggested by another recent study in *The EMBO Journal* (Saeki *et al.*, 2009), and Poh1 could allow recycling of ubiquitin. Undoubtedly, further experiments will follow this exciting biochemical study to unravel the cellular pathways regulated by these surgical chain saws, the K63-specific JAMM-DUBs.

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