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Structural Plasticity Allows UCH37 to Be Primed by RPN13 or Locked Down by INO80G

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Abstract

Two studies in this issue of *Molecular Cell*, VanderLinden et al. (2015) and Sahtoe et al. (2015),report crystal structures that define how deubiquitinating enzyme UCH37 is switched on or off by proteasome ubiquitin receptor RPN13 or chromatin remodeler component INO80G.

Ubiquitination plays a defining role in numerous cellular events and is a well-known signature for triggering protein degradation by the proteasome. Deubiquitinating enzymes (DUBs) disassemble ubiquitin chains, making them integral to the dynamic regulatory roles assumed by ubiquitin and, thus, in need of stringent regulation. The essential cysteine protease UCH37 (UCH-L5) is controlled by its binding partners: it is activated by proteasome ubiquitin receptor RPN13 (ADRM1) or inhibited by chromatin remodeling complex component INO80G (NFRKB). These two companions share a conserved DEUBiquitinase ADaptor (DEUBAD) domain (Sanchez-Pulido et al., 2012) that binds to a unique C-terminal region in UCH37, termed the UCH37-like domain (ULD). In this issue, Sahtoe et al. (2015) and VanderLinden et al. (2015) report independently solved crystal structures of UCH37 complexed with either the RPN13 (with and without ubiquitin) or INO80G DEUBAD domains to reveal how these two adaptor proteins control UCH37 activity.

RPN13 and INO80G DEUBAD domains were found to adopt strikingly different 3-dimensional structures when bound to the ULD (Figure 1) (Sahtoe et al., 2015; VanderLinden et al., 2015). Previously, it was shown that RPN13 DEUBAD domain folds into a compact 8-helical bundle in the apo state (Chen et al., 2010; Jiao et al., 2014). Sahtoe et al. (2015) and VanderLinden et al. (2015) demonstrate that this structure fractures to engulf the UCH37 ULD (Figure 1). The two most C-terminal UCH37 helices occupy a position similar to that of the first two RPN13 DEUBAD helices in the apo state, thereby replacing critical hydrophobic core interactions with intermolecular ones. Deviation between the geometry of the apo and UCH37-compexed state is largest for the N-terminal half of the RPN13 DEUBAD, which in an earlier study also exhibited increased high frequency motions in the free, single-domain fragment (Chen et al., 2010). These N-terminal motions may facilitate exchange to the opened UCH37-bound state.

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In a previously solved crystal structure of UCH37 with a ubiquitin-based suicide inhibitor (Morrow et al., 2013), the extended C-terminal region of ubiquitin was found to snake along a canal that leads to the catalytic cysteine of UCH37 while the ubiquitin loop that contains L8 contacts a UCH pocket more remote from the catalytic site. Ubiquitin is spacially accommodated by partial melting of a UCH domain helix and Sahtoe et al. (2015) and VanderLinden et al. (2015) find that this state is also adopted when UCH37 is bound to RPN13. UCH enzymes have a characteristic active site crossover loop that when shortened inhibits hydrolyase activity (Zhou et al., 2012). This loop was not observed in apo UCH37 (PDB 3IHR) or when bound to ubiquitin (Morrow et al., 2013; Sahtoe et al., 2015; VanderLinden et al., 2015), suggesting that it is flexible in these states. Sahtoe et al. (2015) and VanderLinden et al. (2015) find that a portion of this crossover loop becomes visible when bound to the RPN13 DEUBAD domain and that this interaction directs the loop away from the UCH37 catalytic site. Thus, the RPN13 DEUBAD domain stabilizes a UCH37 state with an accessible active site that is primed to interact with ubiquitin (Sahtoe et al., 2015; VanderLinden et al., 2015).

The structure of apo INO80G DEUBAD has not yet been reported; however, in this issue, Sahtoe et al. (2015) and VanderLinden et al. (2015) solve this DEUBAD structure bound to UCH37 and find it to have six helices, instead of the eight observed in the RPN13 DEUBAD (Chen et al., 2010; Jiao et al., 2014). The three most C-terminal helices of the RPN13 DEUBAD coalesce in the INO80G DEUBAD to a contiguous helix that sweeps across the UCH catalytic domain (Figure 1). To accommodate this extended DEUBAD structure, the second ULD helix in UCH37 bends, changing the orientation of the last two helices relative to the RPN13- and ubiquitin-bound states. Thus, UCH37 also exhibits conformational plasticity in these complexes. The extensive contacts formed between the long INO80G helix and the UCH37 catalytic domain situates a unique short hairpin in this DEUBAD against the pocket used to contact ubiquitin L8. This positioning sterically occludes ubiquitin binding to the UCH domain; mutation or truncation of this INO80G hairpin relieves inhibition and its inclusion in RPN13 DEUBAD abolishes activation (Sahtoe et al., 2015).

The ubiquitin- and proteasome-binding RPN13 pleckstrin-like receptor for ubiquitin (PRU) domain interacts intramolecularly with the DEUBAD, reducing RPN13 affinity for ubiquitin (Chen et al., 2010). Proteasome activates RPN13 for ubiquitin by breaking the PRU:DEUBAD interaction (Chen et al., 2010), and this effect may also be imparted by UCH37, which similarly binds to a surface that overlaps with RPN13 interdomain contacts. Q337 and Q338 are partially exposed in the single DEUBAD domain, and interaction with PRU, which preserves the DEUBAD 8-helical bundle, further sequesters these two glutamines (Chen et al., 2010). They become accessible, however, after DEUBAD reconfiguration by UCH37 and, as occupants of the helix that binds the UCH37 activation loop, positioned to make contact with ubiquitin (Sahtoe et al., 2015; VanderLinden et al., 2015). Their interaction with ubiquitin is not essential for RPN13 activation of UCH37 for ubiquitin-AMC, as their mutation to alanine produced a 2.5-fold reduction (VanderLinden et al., 2015) or negligible effect (Sahtoe et al., 2015); wild-type RPN13 increases this activity 8-fold (Sahtoe et al., 2015; VanderLinden et al., 2015). It is possible, however, that they make a larger contribution when the substrate is a ubiquitin chain by helping to orient the

configuration of their helical residence to optimize interaction with the activator loop and neighboring ubiquitin.

UCH37 activity appears to be limited for diubiquitin hydrolysis, and this is not improved by RPN13 (VanderLinden et al., 2015). It was shown many years ago that UCH37 hydrolyzes ubiquitin moieties from the chain end that is distal to substrate (Lam et al., 1997), whereas RPN13 PRU prefers to be at a K48 linkage site (Schreiner et al., 2008) and, thus, a more proximal ubiquitin. The UCH37 active site is probably too crowded to accommodate its interaction with distal ubiquitin while RPN13 PRU binds to the neighboring proximal ubiquitin of a diubiquitin chain. Thus, RPN13 PRU is more likely to serve as a competitive inhibitor for K48-linked diubiquitin, restricting it from UCH37. It is likely to contribute, however, to UCH37 hydrolysis of longer ubiquitin chains by allowing UCH37 to bind to the distal ubiquitin, while RPN13 PRU binds to a ubiquitin spaced further away (Figure 1). In this capacity, it could assist with substrate recruitment, ubiquitin placement at the cleavage site, and product release. Future studies are needed to dissect the contribution of the RPN13 PRU on UCH37 hydrolysis of ubiquitinated substrates and whether further activation is imparted by their proteasome neighbors.

UCH37 and RPN13 are upregulated in a variety of human cancers, and their covalent targeting by chalcone-based derivatives b-AP15 (D'Arcy et al., 2011) and RA190 (Anchoori et al., 2013), respectively, triggers apoptosis in mammalian cancer cell lines and restricts tumor growth in mice xenografts models (Anchoori et al., 2013; D'Arcy et al., 2011). The papers from VanderLinden et al. (2015) and Sahtoe et al. (2015) open new therapeutic avenues for inhibiting UCH37, with the possibility of designing INO80G small molecule mimics that offer greater specificity than those that solely target the active site cysteine.

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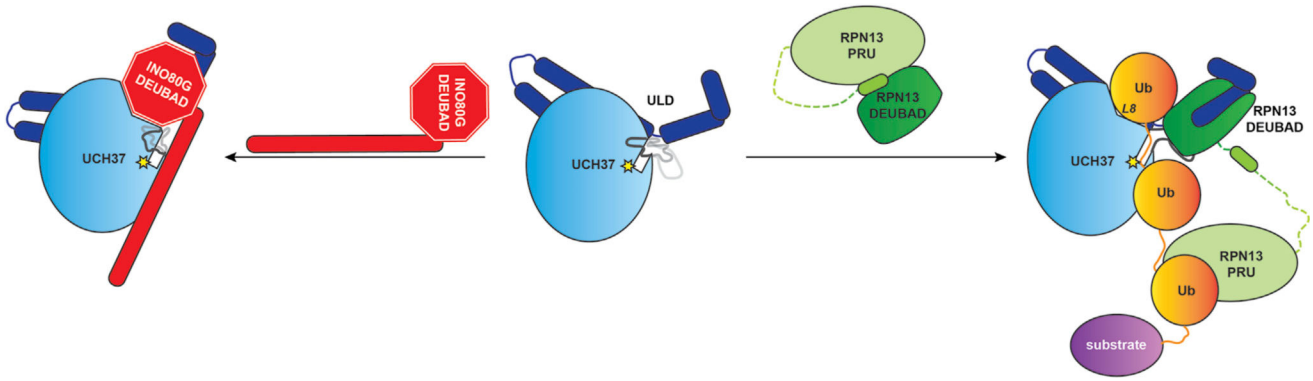

Figure 1. Schematic Highlighting How UCH37 Hydrolase Activity Is Controlled by DEUBAD Regulators RPN13 and INO80G

UCH37 (blue) has a unique C-terminal ULD (indigo) with conformational plasticity that enables binding to divergent DEUBAD domain configurations of RPN13 (green) and INO80G (red).RPN13 DEUBAD (dark green) forms a compact structure that fractures to wrap around the ULD, promoting contact with the UCH37 activation loop (gray) and UCH37-bound ubiquitin (orange). The C-terminal end of ubiquitin slithers along a groove that leads to the catalytic cysteine (yellow star), but in the apo state, the activation loop (gray) is expected to be flexible and restrict access to the catalytic site.RPN13 DEUBAD binds this loop and directs it away from the catalytic site. RPN13 PRU (light green):DEUBAD interaction is likely broken by UCH37, releasing PRU for ubiquitin. The catalytic region is probably too crowded to accommodate PRU when bound to diubiquitin; however, in longer chains it would bind to a proximal ubiquitin, thereby facilitating substrate recruitment. UCH37 has an additional satellite site poised to bindL8 of ubiquitin as its C terminus extends to the catalytic site. INO80G DEUBAD monopolizes this region and uses the C-terminal third of its DEUBAD to form an extended helix that runs along the catalytic domain, creating rearrangements that shut down the active site cleft.