Review

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Layers of DUB regulation

Danny D. Sahtoe and Titia K. Sixma

Division of Biochemistry and Cancer Genomics Center, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, The Netherlands

Proteolytic enzymes, such as (iso–)peptidases, are potentially hazardous for cells. To neutralize their potential danger, tight control of their activities has evolved. Deubiquitylating enzymes (DUBs) are isopeptidases involved in eukaryotic ubiquitylation. They reverse ubiquitin signals by hydrolyzing ubiquitin adducts, giving them control over all aspects of ubiquitin biology. The importance of DUB function is underscored by their frequent deregulation in human disease, making these enzymes potential drug targets. Here, we review the different layers of DUB enzyme regulation. We discuss how post-translational modification (PTM), regulatory domains within DUBs, and incorporation of DUBs into macromolecular complexes contribute to their activity. We conclude that most DUBs are likely to use a combination of these basic regulatory mechanisms.

DUB regulation: background and overview

Conjugation of ubiquitin and ubiquitin-like molecules (Ubl) (Box 1) to lysines of target proteins represents a major type of PTM that regulates countless processes in eukaryotes [1]. These modifications are catalyzed by an enzymatic cascade involving E1 activating enzymes, E2 conjugating enzymes, and E3 ligases (Box 2). Many different types of Ub/Ubl modification exist, because targets can be monoubiquitylated or modified with a variety of polyubiquitin chains (Box 3) that can each have different signaling outcomes.

Ubiquitin signals have profound cellular effects and, therefore, conjugation events are kept in check by ubiquitin deconjugation. This function is performed by a specialized class of isopeptidases called DUBs, which hydrolyze the isopeptide bond between ubiquitin and the target proteins [2,3]. Five different DUB families have been identified: ubiquitin C-terminal hydrolase (UCH), ubiquitin specific protease (USP), ovarian tumor (OTU), Machado-Joseph disease (MJD); and Jab1/Mpn/Mov34 (JAMM) (Box 4). All of these are cysteine isopeptidases except the JAMM family members, which have metalloisopeptidase activity [3].

Due to their critical role in cellular functions, deregulation of enzymes of the ubiquitin system is important in cancer, infectious, and neurological diseases [4–6]. Hence, there is an increasing interest in targeting these molecules pharmaceutically. Given that E2 conjugating enzymes and most E3 ligases lack distinct catalytic clefts, approaches to therapeutic intervention currently focus on DUBs [7].

In the cell, the activity of degrading enzymes is carefully controlled. This has long been known for peptidases, the distant cousins of DUBs, which are tightly regulated not only through production as inactive enzymes (zymogens), but also through proteinaceous inhibitors and elaborate activation cascades to prevent aberrant proteolysis [8]. This tight control is essential, because unscheduled activation can be disastrous for the cell. It is gradually becoming clear that this is also true for DUB isopeptidases. The need to regulate DUB activity can be explained by the large number of ubiquitin conjugates in cells. Without proper regulation, DUBs could unspecifically hydrolyze any ubiquitin conjugate that they encounter, potentially deregulating cellular physiology.

To cope with this, cells have adopted several strategies to ensure that DUB activity is channeled to the right locations at the right time. Some of this regulation occurs at the transcriptional level, but the proteins themselves are regulated in many different ways. A clear understanding of these processes is important for our knowledge of ubiquitin biology and will assist in the development of therapeutic agents targeting specific DUBs. In recent years, insights into DUBs whose catalytic activity is regulated have steadily increased; through advances in the cellular physiology, biophysics, and structural biology of DUBs, we are starting to elucidate the intricate mechanisms that underlie DUB regulation.

The general roles of DUBs and their target and chain specificity have been discussed elsewhere [3,9,10]. Here, we discuss the emerging themes in regulation of DUBs at the protein level. We distinguish different 'layers' of DUB regulation and describe how they affect activity (Figure 1).

Box 1. Ubiquitin and ubiquitin-like molecules

Eukaryotes have a diverse repertoire of PTMs to fine-tune or alter molecular processes. Among these is ubiquitination, where the small 76-amino acid protein ubiquitin is attached to target proteins [1]. Ubiquitin is characterized by a globular β-grasp fold followed by an extended tail harboring a Gly-Gly motif required for conjugation to target proteins. Over the past few decades, other small proteins that share these characteristics with ubiquitin have been identified. Among these are NEDD8, SUMO-1, SUMO-2, SUMO-3, ATG8, ISG15, and FAT10, but more have been identified that can be conjugated to proteins to alter their fate or function. Moreover, the enzymes responsible for their conjugation and deconjugation are also homologous to the enzymes from the ubiquitin system and follow similar mechanisms. Given these commonalities with ubiquitin, these proteins are collectively called Ubl.

Corresponding author: Sixma, T.K. (t.sixma@nki.nl).

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Box 2. Ubiquitin conjugation and deconjugation

The conjugation of ubiquitin to target proteins proceeds via a conserved enzymatic cascade (Figure I) [1]. This cascade results in the formation of an isopeptide bond between the C terminus of ubiquitin and the ε-amino group of a lysine on the target protein. In the first step, an E1-activating enzyme activates the C-terminal carboxyl of ubiquitin and transfers it to its active site cysteine, after which an E2-conjugating enzyme binds the E1 and is 'charged' with ubiquitin at its active site cysteine. E3 ligases next orchestrate the final formation of the isopeptide bond between ubiquitin and target. DUBs can reverse ubiquitin conjugation by catalyzing the hydrolysis of the isopeptide bond (Figure I) [2]. This allows these enzymes to control all aspects of ubiquitin biology.

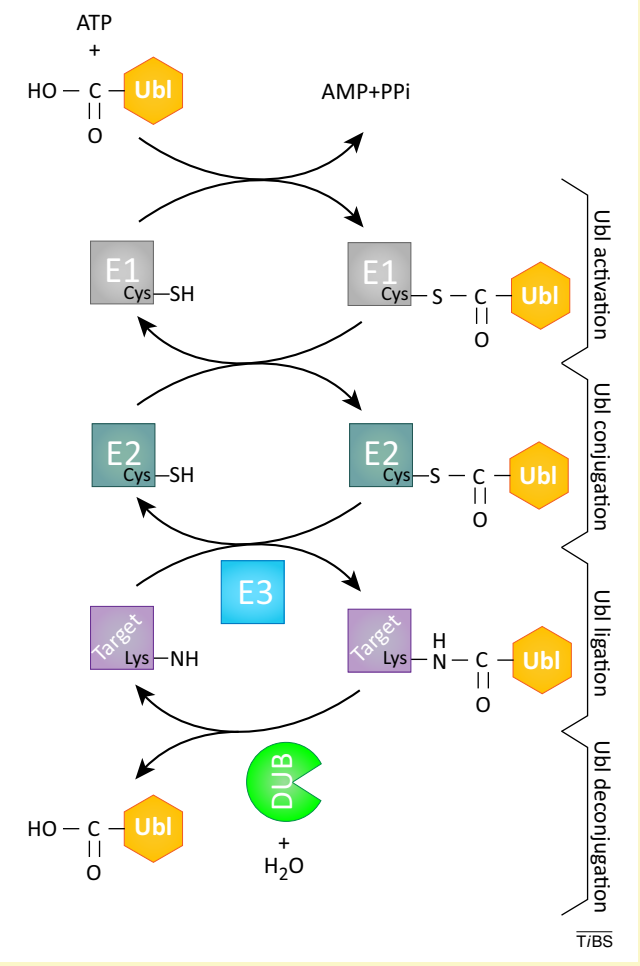

Figure I. Ubl conjugation and deconjugation cascade. Abbreviation: Ubl, ubiquitin-like molecule.

Box 3. Ubiquitin signals

Ubiquitin signals come in many flavors, some of which are schematically depicted in Figure I. Target proteins can be conjugated with a single ubiquitin or multiple ubiquitins. Furthermore, ubiquitin can also be conjugated to itself in eight different ways because it has seven lysine and one free amino-group of the Met1 amino acid that all can serve as targets. Thus, different ubiquitin chains are possible. These chains have different structural properties and are associated with different cellular processes [93]. For example, proteins modified with polyubiquitin chains linked through lysine 48 mark them for proteasomal destruction, whereas lysine 63 and 'linear' Met1-linked chains have roles in signaling pathways. Mixed chains, containing several different linkages in the same polyubiquitin molecule, have also been reported [94]. Ubl-deconjugating enzymes can hydrolyze polyubiquitin chains to single ubiquitin moieties with, in some cases, remarkable specificity. For example, the DUB OTULIN can only hydrolyze linear Met1-linked ubiquitin chains [33], while the DUB AMSH specifically cleaves Lys63-linked polyubiquitin chains [55].

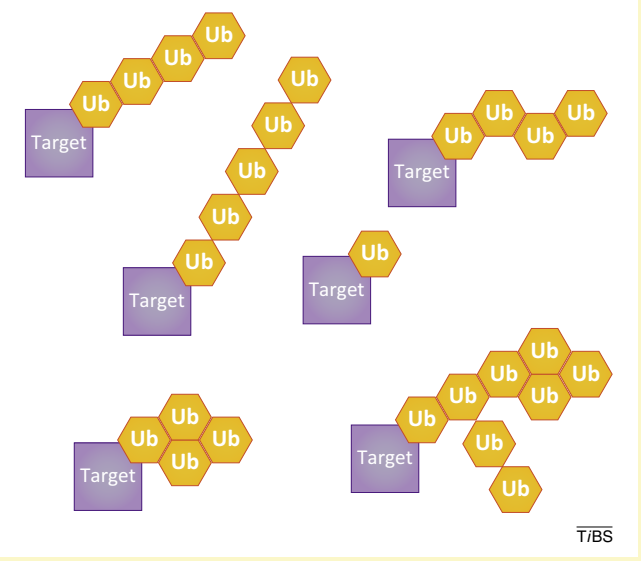

Figure I. Different ubiquitin chain types result in different signals.

After examination of the individual layers, we analyze how these different mechanisms can cooperate. Although our list of examples is not exhaustive (Table 1), it provides a good basis for discussing the different layers of DUB regulation.

Cellular and target recruitment

In Figure 1, we present a simplified classification of the different layers of DUB regulation. The first layer we discuss is that of DUB recruitment factors. Guiding the almost 100 DUBs encoded in the human genome to their relevant substrates and pathways is crucial for cellular physiology because it insulates DUBs from unwanted interactions and the cell from spurious activity. It can be mediated by distinct regions within the enzyme or by external factors: For instance, the Ubl domain of ubiquitin-specific protease 14 (USP14) recruits it to the proteasome, where its activity is increased 500-fold [11]. The endosomal protein signal transducing adaptor molecule (STAM) recruits the DUBs AMSH (associated molecule with an Src3 homology domain of STAM) and USP8 to the endosome pathway by interacting with an SRC homology 3 (SH3)-binding motif or MIT domain (microtubule interacting and transport), respectively [12,13].

Another pathway that requires proper DUB recruitment is the DNA damage response (DDR). After ultraviolet (UV)-induced DNA damage, monoubiquitylated proliferating cell nuclear antigen (PCNA) mediates signaling that leads to repair. The DUB complex USP1/USP1-associated factor 1 (UAF1) deubiquitylates PCNA after the complex is recruited to the substrate by recruitment factor human ELG1 [14]. BRCA1/BRCA2-containing complex, subunit 36 (BRCC36) is another DUB in the DDR, where it deubiquitylates several proteins as a catalytic subunit of the BRCA1-A complex. In this complex, specialized ubiquitin and small ubiquitin-like modifier (SUMO)-binding domains recruit BRCC36 to sites of

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