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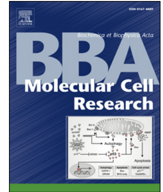

Review

Regulation of proteolysis by human deubiquitinating enzymes ☆

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ABSTRACT

The post-translational attachment of one or several ubiquitin molecules to a protein generates a variety of targeting signals that are used in many different ways in the cell. Ubiquitination can alter the activity, localization, protein–protein interactions or stability of the targeted protein. Further, a very large number of proteins are subject to regulation by ubiquitin-dependent processes, meaning that virtually all cellular functions are impacted by these pathways. Nearly a hundred enzymes from five different gene families (the deubiquitinating enzymes or DUBs), reverse this modification by hydrolyzing the (iso)peptide bond tethering ubiquitin to itself or the target protein. Four of these families are thiol proteases and one is a metalloprotease. DUBs of the Ubiquitin C-terminal Hydrolase (UCH) family act on small molecule adducts of ubiquitin, process the ubiquitin proprotein, and trim ubiquitin from the distal end of a polyubiquitin chain. Ubiquitin Specific Proteases (USPs) tend to recognize and encounter their substrates by interaction of the variable regions of their sequence with the substrate protein directly, or with scaffolds or substrate adapters in multiprotein complexes. Ovarian Tumor (OTU) domain DUBs show remarkable specificity for different Ub chain linkages and may have evolved to recognize substrates on the basis of those linkages. The Josephin family of DUBs may specialize in distinguishing between polyubiquitin chains of different lengths. Finally, the JAB1/MPN+/MOV34 (JAMM) domain metalloproteases cleave the isopeptide bond near the attachment point of polyubiquitin and substrate, as well as being highly specific for the K63 poly-Ub linkage. These DUBs regulate proteolysis by: directly interacting with and co-regulating E3 ligases; altering the level of substrate ubiquitination; hydrolyzing or remodeling ubiquitinated and poly-ubiquitinated substrates; acting in specific locations in the cell and altering the localization of the target protein; and acting on proteasome bound substrates to facilitate or inhibit proteolysis. Thus, the scope and regulation of the ubiquitin pathway is very similar to that of phosphorylation, with the DUBs serving the same functions as the phosphatase. This article is part of a Special Issue entitled: Ubiquitin–Proteasome System.

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1. Ubiquitination is a post-translational targeting signal

Ubiquitin (Ub) is a highly conserved 76-residue protein present in all eukaryotic cells. Through a series of enzymatic reactions, the C-terminus of Ub becomes activated and conjugated to the ε-amino group of lysine or the N-terminal α-amino group of another Ub, forming poly-Ub chains, or conjugated to target proteins to form a ubiquitinated protein [1]. The conjugation pathway begins with an E1 activating enzyme that uses ATP to first adenylate Ub's C-terminal carboxylate and transfer it to an E2 conjugating enzyme (~35 in humans) forming an E2-Ub thioester intermediate (E2 ~ Ub) [2,3]. E3 Ub ligases (>500 putative E3s in humans) provide substrate specificity within the conjugation pathway by selectively binding both E2 ~ Ub and the target protein to catalyze the transfer of Ub to a lysine or α-amino group of the target protein. E3s fall into two general categories, RING domain E3s (Really Interesting New Gene) which catalyze direct transfer of the E2 ~ Ub to a lysine group within substrate/Ub, and HECT (homologous to E6AP Carboxyl-terminus) domain or RBR (RING-between-RING) E3s which contain active site thiols and form an additional E3 ~ Ub thioester intermediate prior to ligation onto Ub/substrate [1,4–6].

Ubiquitination can generate many different types of covalent modifications [7]. The conjugation of a single Ub to a protein amino group is called mono-ubiquitination. This modification was first described when the chromosomal protein A24 was identified as histone H2A mono-ubiquitinated at K119 [8]. This histone modification is an epigenetic mark that regulates chromosome structure and transcription [9], while mono-ubiquitination of many cell surface receptors is used as a sorting signal to direct these endocytosed proteins to lysosomal degradation [10]. Poly-Ub chains can be assembled when additional ubiquitins are conjugated to the protein-bound monoubiquitin using any of the seven lysines within Ub or the N-terminal α-amino group (forming linear poly-Ub). Thus, ubiquitination of proteins can result in many structurally unique polymers that direct the modified proteins to different fates. Proteins modified with poly-Ub chains linked through K48 or K11 of Ub are recognized and degraded by the 26S proteasome, while

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K63 poly-Ub functions in regulating other cellular processes such as signal transduction, lysosome-directed protein sorting and the DNA damage response [10–14]. Linear poly-Ub is assembled during inflammatory signaling [15,16]. Thus, Ub is a post-translational modification similar to phosphorylation or glycosylation and regulates the stability, localization, or activity of modified proteins. DUBs play a role very similar to that of the phosphatases in kinase/phosphatase pathways.

It is worth noting that this system of modification is so useful to the cell that several other Ub-like proteins have evolved. Thus, Ub-like proteins such as Nedd8, SUMO, and others undergo virtually identical activation and conjugation reactions to modify a large number of proteins [17,18]. A separate family of DUBs containing the ULP (Ubiquitin-like protease) domain exhibit specificity for SUMOylated proteins [19]. This review will concentrate on Ub-dependent processes but will briefly mention Nedd8 modifications since it is required for optimal activity of one family of E3 ligases.

Like all regulatory post-translational modifications, ubiquitination is reversible. A class of proteases called deubiquitinating enzymes (DUBs) removes Ub from target proteins and disassembles polyubiquitin chains [20,21]. Deubiquitination is the process of hydrolyzing the (iso)peptide bond linking Ub to a substrate or to itself in a poly-Ub chain. Most often the bond hydrolyzed is an isopeptide linkage between a lysine ε-amino group and the C-terminal carboxylate of Ub. Some DUBs display specificity toward different chain linkages, such as K48 or K63 poly-Ub, while some act less specifically and are capable of cleaving multiple chain types or even Ub-like modifiers [20]. Like many other proteases, DUBs are often inactive or autoinhibited, remaining inactive until they are recruited to their site of activity or bind to the proper substrates. To achieve proper localization and specificity DUBs are modular, requiring domains outside the catalytic core to associate with scaffolds, substrate adapters, or the substrates themselves [20].

This review will discuss several of these deubiquitinating enzymes and highlight a number of ways in which they can regulate proteolysis and other Ub-dependent processes (Fig. 1). It is not comprehensive, but only exemplary of the different modes of action observed to date. We will concentrate on those DUBs that have been extensively characterized, where structures are known, and where their mechanisms of action highlight different aspects of cellular regulatory strategies.

2. The five families of deubiquitinating enzymes

An early bioinformatics approach identified 95 putative DUBs in the human genome [22], yet several lack an active site cysteine or have been shown to act on Ub-like protein conjugates. A more recent estimate puts the number of human ubiquitin-specific DUBs at 86 [23]. DUBs can be grouped into five families based on their conserved catalytic domains. Four of these families are thiol proteases and comprise the bulk of DUBs, while the fifth family is a small group of Ub specific metalloproteases (see below).

2.1. Thiol protease DUBs

Most DUBs are thiol proteases that utilize a catalytic mechanism analogous to that of the plant cysteine protease papain [24,25]. Thiol-containing DUBs contain a Cys-His-Asp/Asn catalytic triad in which the Asp/Asn functions to polarize and orient the His, while the His serves as a general acid/base by both priming the catalytic Cys for nucleophilic attack on the (iso)peptide carbonyl carbon and by donating a proton to the lysine ε-amino leaving group. The nucleophilic attack of the catalytic Cys on the carbonyl carbon produces a negatively charged transition state that is stabilized by an oxyanion hole composed of hydrogen bond donors. A Cys-carbonyl acyl intermediate ensues and is then hydrolyzed by nucleophilic attack of a water molecule to liberate a protein C-terminal carboxylate and regenerate the enzyme. A striking feature of the thiol protease DUBs is that despite divergent tertiary folds, crystal structures in complex with Ub have revealed the positions of the catalytic dyad/triad discussed above are nearly superimposable [21,26]. Upon binding Ub, the catalytic domains often undergo structural rearrangements to order regions involved in catalysis. Recently it has been found that many DUBs are inactivated by oxidation of the catalytic cysteine to sulphenic acid (−SOH) [27–29]. The sulphenic acid can be further oxidized to generate sulphinic acid ($-SO_2H$), sulphonic acid ($-SO_3H$), a disulfide, or a sulphenyl amide, which occurs when a sulphenic acid reacts with a nearby backbone amide. Like the disulfide bond, the suphenic acid and sulphenyl amide forms can be reduced with DTT or glutathione.

The thiol proteases are reversibly inhibited by Ub C-terminal aldehyde, forming a thio-hemiacetal between the aldehyde group and the active site thiol. They are irreversibly inactivated by alkylation or oxidation of the catalytic cysteine or reaction of the active site thiol on Ub derivatives containing electrophilic groups near the C-terminus of Ub (i.e., Ub-vinylsulfone, -vinylmethyl ester, -chloroethylamine, and more recently -propargylamine)[30–34].

2.1.1. Ub C-terminal Hydrolase (UCH) domain

DUBs of the UCH family are thiol proteases that contain an N-terminal, 230-residue catalytic domain, sometimes followed by C-terminal extensions that mediate protein-protein interactions. In humans there are four UCH DUBs (UCH-L1, UCH-L3, UCH37/UCH-L5, and BAP1) and these can be sub-grouped based on their substrate specificity. *The smaller UCH DUBs (UCH-L1 and UCH-L3) prefer cleaving small leaving groups from the C-terminus of ubiquitin, while the larger UCH DUBs (UCH37 and BAP1) can disassemble poly-Ub chains.*

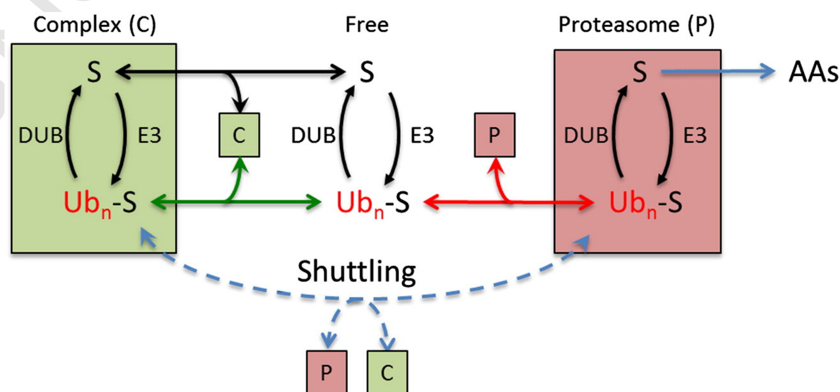

Fig. 1. A given substrate (S) can exist in the free state or bound in a complex (C), each location containing its own distinct E3 and DUB. The strength of binding can be different for the free (black line) or ubiquitinated (green line) substrate. Depending on the value of these binding constants, ubiquitination can either recruit or release S from C. Ubiquitinated protein can be directly bound by the proteasome (red line) or shuttled to the proteasome (dashed blue line) if C is a ubiquitin receptor.

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