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ORIGINAL PAPER

PTMs in Conversation: Activity and Function of Deubiquitinating Enzymes Regulated via Post-Translational Modifications

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Abstract Deubiquitinating enzymes (DUBs) constitute a diverse protein family and their impact on numerous biological and pathological processes has now been widely appreciated. Many DUB functions have to be tightly controlled within the cell, and this can be achieved in several ways, such as substrate-induced conformational changes, binding to adaptor proteins, proteolytic cleavage, and post-translational modifications (PTMs). This review is focused on the role of PTMs including monoubiquitination, sumoylation, acetylation, and phosphorylation as characterized and putative regulative factors of DUB function. Although this aspect of DUB functionality has not been yet thoroughly studied, PTMs represent a versatile and reversible method of controlling the role of DUBs in biological processes. In several cases PTMs might constitute a feedback mechanism insuring proper functioning of the ubiquitin proteasome system and other DUB-related pathways.

Keywords Ubiquitin · Protease · Post-translational modification · Phosphorylation · Acetylation · Ubiquitination · Deubiquitination · Deubiquitinating enzymes

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Abbreviations

UCH	Ubiquitin *C*-terminal hydrolase
USP	Ubiquitin-specific protease
OTU	Ovarian tumor domain
PTM	Post-translational modification

Introduction

The human genome encodes for approximately 80 putative deubiquitinating enzymes (DUBs), including cysteine proteases and several metalloproteases [1]. The diverse functions that DUBs play within the cell can be classified into three major categories. Firstly, DUBs process linear polyubiquitin precursor proteins, such as ribosomal fusion proteins, into single ubiquitin molecules (reviewed in [2]). Secondly, DUBs recycle ubiquitin by processing polyubiquitin chains to generate free ubiquitin that can subsequently enter the ubiquitin pool for subsequent ubiquitin conjugation events. This is a critical process since free polyubiquitin chains can inhibit the binding of polyubiquitinated substrates to the 26S proteasome competitively [3–5]. Finally, DUBs remove ubiquitin from ubiquitinated substrates, antagonizing ubiquitin conjugation by E3 ligases [6, 7]. The vast number of DUBs belonging to five distinct protein families suggests that there is a specialization in terms of their function and specificity. Indeed, it has been demonstrated that DUBs target distinct pathways and their localization may be limited to certain subcellular compartments [1, 8]. Moreover, many DUBs have been linked to pathological conditions, underlying their physiological significance in health and disease (reviewed in [9]).

Modes of Regulation of DUB Activity

Since the catalytic activity of DUBs is so specific and in many cases functionally critical, one would anticipate multiple mechanisms of its control, and several ones have been already described (reviewed in [10]). DUBs are generally expressed as active enzymes, rather than inactive precursors. However, certain DUBs require ubiquitin binding to obtain their active conformation and that prevents their uncontrolled proteolytic activity. The structural data for several DUBs reveal that ubiquitin-binding by DUBs is accompanied by active site rearrangements and that such conformational alterations induce their hydrolytic activity, which has been demonstrated for OTUB1, UCH-L1, UCH-L3, USP7, USP14, and *S. cerevisiae* YUH1 [11–19]. Another way of modulating DUB activity is through the binding of scaffold and adaptor proteins. Some DUBs display low affinity for ubiquitin and therefore require additional interactors for binding ubiquitinated substrates efficiently [20]. DUBs may require to be incorporated into large macromolecular complexes to attain the active state, exemplified by USP14 or POH1 that are activated by their binding to the 26S proteasome complex [10, 21, 22]. Activation of USP8 and AMSH is facilitated by signal transducing adaptor molecule 2 (STAM2), and both proteins are involved in regulating endocytic trafficking [23]. Protein–protein interactions can also inhibit protease activity, for example UCH37 function is inhibited by its binding to the chromatin-remodeling complex [24]. Proteolytic cleavage of DUBs is another way of regulation of their function. This is exemplified by USP1, which undergoes autoproteolysis that in turn inactivates this enzyme [25]. Last but not least, many DUBs are subjected to post-translational modifications (PTMs), possibly representing an effective and reversible means of regulating their activity or function. This review will discuss the documented examples of the PTMs in DUBs and their various phenotypic consequences (summarized in Table 1).

Phosphorylation of CYLD in the NF-κB Pathway

The ubiquitin-specific protease involved in cylindromatosis (CYLD) is one of the best studied examples of post-translationally modified DUBs. CYLD specifically cleaves Lys^{63}-linked polyubiquitin chains and acts on TRAF2, TRAF6, and several other substrates, which results in negative regulation of the NF-κB pathway ([26–28], reviewed in [29]). CYLD is a tumor suppressor and an important player in the host defense mechanisms against bacterial infection, as shown for several pathogens [30–33]. CYLD becomes phosphorylated as a response to treatment with a number of NF-κB-inducing factors, such as LPS or TNF-α [34]. This transient modification occurs at several sites in a region located within close proximity to the TRAF2-binding site, which includes Ser^{418}. The biochemical analysis using phosphomimetic mutants demonstrated that this PTM negatively affects the deubiquitinating activity of CYLD on TRAF2, most likely through interfering with the catalytic activity of CYLD, since the binding of TRAF2 to a CYLD mutant mimicking phosphorylation on Ser^{418} is not affected (Fig. 1a; [34]). There is some initial evidence that IKKγ (I kappa B kinase gamma) mediates CYLD phosphorylation on Ser^{418} [34], although a more recent report suggests that IKKε (I kappa B kinase epsilon) is a much more efficient kinase for this site [35]. Interestingly, IKKα (I kappa B kinase alpha) and IKKß (I kappa B kinase beta) are also able to phosphorylate CYLD in vitro, although in vivo they require additional assistance of IKKγ. In addition to down-regulation of the NF-κB pathway [34], CYLD phosphorylation has been demonstrated to have a physiological relevance in increasing cell transformation [35], hence precise identification of a kinase or a kinase cascade involved in this process might provide potential targets for pharmacological intervention strategies in the treatment of cancer.

Phosphorylation-Regulated Activity of A20

A20 is an ovarian tumor domain (OTU)-containing protease with a well-defined function in pro-inflammatory events. It down-regulates activation of the transcription factor NF-κB and therefore plays an important role in inflammation [36–38]. Interestingly, next to the OTU domain involved in cleavage of Lys^{63}-linked polyubiquitin chains from the protein substrates TRAFs, RIPs and NEMO, it also contains the *C*-terminal zinc finger domain that acts as a ubiquitin ligase and is responsible for building Lys^{48}-linked polyubiquitin conjugates on RIPs, thus targeting them to the proteasome [39, 40]. Therefore, A20 has a dual, or editing function on its substrates, removing one type of polyubiquitin chain and attaching another. A positional scanning peptide library technique combined with a bioinformatics approach identified A20 as a putative substrate for the IKKß kinase. Mass spectrometric analysis mapped the phosphorylation site to Ser^{381} that was verified in vitro and in vivo. IKKß-mediated A20 phosphorylation has been shown to increase its activity toward NEMO, thereby further down-regulating the NF-κB pathway. It is not conclusive, however, whether phosphorylation on Ser^{381} affects the E3 ubiquitin ligase or deubiquitinase activity of A20, although the modification occurs at the zinc finger domain of the protein, so the former would be expected [41].

Table 1 Review of characterized post-translational modifications (PTMs) of deubiquitinating enzymes (DUBs)

DUB	Modification	Residue	Domain	Modifying enzyme	Physiological effect	References
A20	Phosphorylation	Ser381	Catalytic region	I kappa B kinase beta	Increased A20-mediated downregulation of NF-κB	[41]
Ataxin-3	Ubiquitination				Enhancement of catalytic activity	[45]
Ataxin-3	Phosphorylation	Ser340, Ser352	UIM	CK2	Influence on nuclear localization, aggregation, and stability	[46]
CYLD	Phosphorylation	Multiple residues within region 447–956, including Ser418	Within close proximity to the TRAF2-binding site	Possibly IkB kinase gamma, alpha or epsilon	Suppression of TRAF2 deubiquitination	[34, 35]
OTUB1	Phosphorylation	Ser16, Ser18, Tyr26	*N*-terminus with unknown function		Suppression of catalytic activity and protein–protein interaction	[50]
Ubp-M (USP16)	Phosphorylation				Phosphorylated form is enzymatically active; phosphorylation is associated with the mitosis and dephosphorylation with the metaphase/anaphase transition	[53, 54]
UCH-L1	*O*-glycosylation				*O*-glycosylated in the nerve terminals	[56]
UCH-L1	Monoubiquitination	Multiple, including Lys4, Lys65, Lys71, Lys157	Within close proximity to the active site		Suppression of catalytic activity by preventing binding to ubiquitinated targets	[57]
USP10	Phosphorylation	Thr42 and Ser337	Thr42 is within the protein–protein interaction domain	ATM	Affected translocation and stabilization	[76]
USP25	Monoubiquitination	Lys99	UIM		Hypothesized activation of catalytic activity	[78]
USP25	Phosphorylation	Tyr740		SYK	Negative effect on protein stabilization	[80]
USP25	Sumoylation	Lys99, Lys141	UIM		Inhibition of catalytic activity	[81]
USP4	Ubiquitination		Ro52 (TRIM21)		Unknown; possibly part of the transregulation mechanism toward Ro52	[121]
USP44	Phosphorylation				Phosphorylation during mitosis	[85]
USP44	Lys48- and Lys63-polyubiquitination					[84]
USP6	Mono-/poly-ubiquitination				Monoubiquitination depends on its association with calcium (Ca^{2+})-binding protein calmodulin (CaM)	[59]
USP7	Phosphorylation	Ser18 and Ser963	Within close proximity to protein–protein interaction domains			[65, 66]
USP7	Ubiquitination	Lys869	Within close proximity to protein–protein interaction domains			[66]
USP8	Phosphorylation	Ser680			Suppression of catalytic activity, alteration of the subcellular localization	[69]
USP8	Phosphorylation	Tyrosine phosphorylation	*N*-terminus			[73]
USP8	Phosphorylation	Thr907		Akt-mediated	Possibly increased protein stability	[74, 75]
USP28	Phosphorylation	Ser67, Ser714		Possibly ATM	Phosphorylated in response to ionizing irradiation	[83]

Information includes the type of a PTM, modified residues, affected domains within a DUB, the modifying enzyme(s) and a physiological effect of the PTM

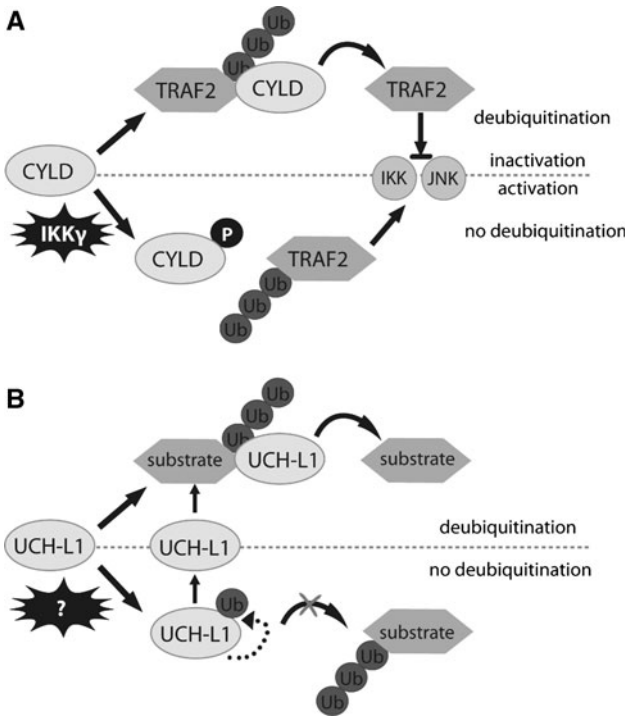

Fig. 1 PTMs in control of DUB activity exemplified by CYLD and UCH-L1. **a** Phosphorylation of CYLD impairs its deubiquitinating activity toward TRAF2. CYLD cleaves Lys^{63}-linked polyubiquitin chains from TRAF2, which results in negative regulation of the NF-κB pathway by inactivation of kinases JNK and IKK. IKKγ-mediated phosphorylation impairs its catalytic activity, in effect contributing to activation of JNK and IKK and positive regulation of NF-κB. **b** Monoubiquitination of UCH-L1 modulates its enzymatic function. UCH-L1 shortens conjugated polyubiquitin chains on the substrate proteins, and monoubiquitination of UCH-L1 hinders this activity by impairing its binding to ubiquitin. UCH-L1 is able to self-regulate its own ubiquitination status through auto-deubiquitination

Post-Translational Modifications Modulate Function of Ataxin-3

Ataxin-3 (AT3) is a polyglutamine disease protein regulating ERAD substrate trafficking to the proteasome. It contains an *N*-terminal Josephin domain [42] and preferentially cleaves Lys^{63}-polyubiquitin chains, displaying even higher activity toward Lys^{63}-ubiquitin linkages that are within mixed linkage ubiquitin chains [43]. AT3 undergoes ubiquitination [44], which increases its ability to process hexa-ubiquitin chains but in the tested conditions it does not alter its specificity to the linkage type [45]. This observation has been made for both wild-type AT3 and the pathogenic AT3 with polyQ expansion causing a neurodegenerative disorder, spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD). Ubiquitination of AT3 can be induced by certain stress factors, including inhibition of the proteasome or treatment with dithiothreitol (DTT) that promotes the unfolded protein response (UPR). It has therefore been proposed that AT3 is regulated by a feedback loop mechanism that helps to restore the homeostasis related to the ubiquitin pathway [45]. Moreover, AT3 is phosphorylated by protein casein kinase 2 (CK2). Phosphorylation occurs within the ubiquitin interacting motif (UIM) of AT3 and is critical for the nuclear localization of normal and pathogenic AT3. Inhibition of AT3 phosphorylation contributes to its decreased translocation to the nucleus and formation of nuclear inclusions. CK2-dependent phosphorylation of AT3 might be crucial in the stress response, because thermal stress has been shown to increase the CK2-modulated nuclear abundance of AT3. Furthermore, phosphorylation might also stabilize AT3, as observed in a pulse-chase experiment using an AT3 mutant mimicking phosphorylation [46].

Otubain 1 Phosphorylation Interferes with its Catalytic Activity and Function in Bacterial Infection

Otubain 1 (OTUB1), a member of OTU-containing protein family, is the only DUB for which specificity for Lys^{48}-ubiquitin linkages has been clearly documented [12, 47]. OTUB1 functions in *T* cell anergy [48, 49], infection with *Yersinia* [50] and in DNA double strand break repair [51]. OTUB1 is predicted to have multiple phosphorylation sites, and three of them have been mapped to Ser^{16}, Ser^{18}, and Tyr^{26} [50]. Phosphomimicry analysis suggests that phosphorylation on these sites influences protein–protein binding and the ability of OTUB1 to react with a ubiquitin-based active-site probe, indicating reduction of its catalytic activity. OTUB1-mediated stabilization of a small GTPase RhoA involved in cytoskeletal alterations has been negatively regulated by phosphorylation, which might be either due to decreased protein–protein binding capabilities or a lower catalytic activity. Finally, the physiological relevance of this modification is highlighted by the fact that OTUB1 phosphomimetic mutants did not influence bacterial invasion, in contrast to the wildtype OTUB1 [50]. The phosphorylation sites are all located in the *N*-terminal part of OTUB1, a domain that has been shown to be critical to exert its function in regulating DNA double strand break repair, indicating a possible regulatory mechanism [51, 52].

Ubp-M Phosphorylation on the Onset of Mitosis

A novel ubiquitin-processing protease Ubp-M (USP16) has been recently identified in the pool of proteins phosphorylated during mitosis [53]. Its function is yet unknown, but it has been postulated that Ubp-M might interfere with cell viability by modifying chromatin functions. The fact that Ubp-M is capable of deubiquitinating histone H2A in vitro is consistent with this hypothesis. Interestingly,

phosphorylation does not interfere with the enzymatic activity of this DUB, but it does correlate with histone H2A deubiquitination during the cell cycle. Ubp-M gets rapidly dephosphorylated during a shift from metaphase to anaphase [53, 54].

Post-Translational Modifications of UCH-L1 Involved in Neurodegenerative Diseases

UCH-L1, a ubiquitin *C*-terminal hydrolase involved in Parkinson's disease and other neurodegenerative disorders (reviewed in [55]), is highly expressed in neurons but its substrates and function have not yet been defined. UCH-L1 is *O*-glycosylated in the nerve terminals, although this modification has not been shown to have any effect on its function [56]. Moreover, UCH-L1 undergoes monoubiquitination at multiple lysines within close proximity to its active site. This PTM appears to control the enzymatic function of UCH-L1 since monoubiquitination impairs its binding to ubiquitin and an ability to increase the monoubiquitin pool in cells, but it has no effect on its localization (Fig. 1b). Importantly, UCH-L1 is able to regulate its own ubiquitination status through auto-deubiquitination, therefore controlling its catalytic capabilities in an autoregulatory feedback loop [57].

Ubiquitination of USP6 in the Context of Protein–Protein Interaction

USP6 (TRE17) is a ubiquitin-specific protease implicated in human neoplasia with unidentified targets for its DUB activity [58]. It has been shown to be mono- and polyubiquitinated, and mono-ubiquitination of USP6 depends on its association with calcium (Ca^{2+})-binding protein calmodulin (CaM). USP6 can promote its own deubiquitination, suggesting a possible mode of auto-regulation, but the physiological relevance of this modification, including the effect on its catalytic activity, remains to be uncovered [59].

USP7—A Deubiquitinase Involved in Tumor Development is Phosphorylated and Ubiquitinated

USP7 (Herpes-associated USP; HAUSP), a DUB described predominantly for its role in cancer biology, is involved in processes such as transcriptional regulation, DNA replication, apoptosis, and possibly in endosomal organization ([60], reviewed in [61, 62]). It interacts with p53, Hdm2 and Hdmx, and its deubiquitinating function towards these proteins protects cells from apoptosis [63, 64]. PTMs documented for USP7 include phosphorylation on Ser^{18} and Ser^{963}, and ubiquitination on Lys^{869}, although any relation of these modifications to its activity has not been demonstrated so far [65, 66]. Ser^{18} is likely to be a target for casein kinase 2 (CK2)-mediated phosphorylation, especially since CK2 co-immunoprecipitates with USP7, suggesting their possible interaction [66]. Both phosphorylation sites of USP7 are located near its protein–protein interaction domains, similarly to the ones of CYLD [34]. It is therefore plausible that this modification might have an effect on USP7 substrates or possibly other protein interactions. Interestingly, the ubiquitination site of USP7 is placed close to the region where it was reported to interact with ICP0, a viral E3 ubiquitin ligase [67], supporting the previous finding that ICP0 targets USP7 for ubiquitination [68].

Role of Phosphorylation Events in the Activity and Stability of USP8

USP8 (UBPY) plays a role in endosomal sorting by deubiquitinating ligand-activated epidermal growth factor (EGFR) on early endosomes [69]. A mass spectrometry-based analysis of the phosphoproteome identified USP8 as an interactor of 14-3-3ε during anaphase, and two independent studies mapped the phosphorylation site to Ser^{680} [70, 71]. This site has been then demonstrated to be critical for the subcellular localization of USP8, and while the wildtype USP8 localizes primarily to the cytosol, the majority of USP8 was found in the nucleus if the Ser^{680} was mutated to alanine [70, 71], but this finding was not supported by another study [72]. Furthermore, the catalytic activity of USP8 is inhibited by phosphorylation on Ser^{680}, based on the fact that the S680A mutant of USP8 exhibites enhanced DUB activity toward polyubiquitin chains and EGFR. This phosphorylation-mediated regulation of USP8 is present during the interphase, while during the M phase USP8 is dephosphorylated [72]. Another study found USP8 to be a substrate for the EGF-activated Src-family tyrosine kinases although its biological significance is not yet understood and the phosphorylation sites mediated by these kinases have not been mapped thus far [73]. USP8 is also phosphorylated by Akt on Thr^{907}, which contributes to its stability [74, 75].

Translocation and Stabilization of USP10 is Mediated by Phosphorylation

USP10 has been recently described as a DUB targeting p53 for polyubiquitin chain cleavage [76]. As mentioned earlier, USP7 is a DUB that deubiquitinates p53 and its E3

ligase Hdm2 [63], but in contrast to USP7, USP10 has been only found to interact with and deubiquitinate p53, and it is predominantly localized in the cytoplasm in unstressed cells, while USP7 is mainly a nuclear protein [76]. Therefore, while USP7 targets p53 in the nucleus, USP10 deubiquitinates cytoplasmic p53 and upon genotoxic stress it translocates to the nucleus to activate p53. ATM phosphorylates USP10 on Thr^{42} and Ser^{337}, and this event is required for the stabilization of USP10 and its translocation into nucleus after DNA damage. The alanine mutation of the Thr^{42}/Ser^{337} has not been shown to interfere with the capability of USP10 to deubiquitinate p53, but it impedes its nucleolar translocation and stabilization, which in effect suppresses USP10-mediated activation of p53 in response to DNA damage [76].

Various PTMs of USP25 and their Effect on its Catalytic Activity

The physiological role of USP25, a member of the USP family [77] remains to be explored. This USP contains a ubiquitin-associated domain (UBA) as well as two ubiquitin binding-domains (UBDs, [78]), and its muscular isoform interacts with three sarcomeric proteins, having a stabilizing effect on one of them, myosin binding protein C1 (MyBPC1; [79]). Recently, the tyrosine kinase SYK has been found to phosphorylate USP25, predictably on the Tyr^{740} residue. The protease activity of USP25 is not affected by SYK-mediated phosphorylation, but it decreases its protein levels, although not due to its increased proteasomal degradation [80]. USP25 is also modified by SUMO-1 and SUMO-2/3, among which the latter PTM has been shown to be more predominant. Sumoylation occurs on Lys^{99} and Lys^{141}, which are located within the ubiquitin-interacting motif (UIM), required for the protease activity of USP25. USP25 sumoylation indeed inhibits the catalytic activity of USP25 imposed by its reduced binding to polyubiquitin chains [81]. Moreover, ubiquitination of muscular isoforms of USP25 has also been detected, and similarly to sumoylation it affected Lys^{99}. Mutation of this residue negatively regulates USP25-mediated stabilization of MyBPC1 and a mutually exclusive modification on Lys^{99}—sumoylation and ubiquitination—might have opposite effects on the enzyme isopeptidase activity. Importantly, USP25 is able to auto-deubiquitinate itself possibly representing a mechanism of auto-regulation [78].

ATM/IR-Dependent Phosphorylation of USP28

A deubiquitinase USP28 binds to the SCF^{Fbw7} ubiquitin E3 ligase, stabilizing Myc, and therefore promoting cell proliferation [82]. Moreover, USP28 binds checkpoint proteins 53BP1, Claspin, and Mdc1 [83]. In response to IR, USP28 becomes phosphorylated on Ser^{67} and Ser^{714} in an ATM-dependent manner [83]. This modification is likely to regulate the complex-formation with the DNA checkpoint proteins, supported by the fact that cell exposure to irradiation induces Myc dissociation from USP28 [82].

Phosphorylation of USP44 during Mitosis

USP44, a predominantly nuclear DUB and an important regulator of the spindle checkpoint, undergoes phosphorylation during mitosis [84]. This step may activate USP44 specifically for the checkpoint arrest, regulated for instance by mitotic cyclin-dependent kinases or spindle checkpoint kinases [85]. Moreover, USP44 is a documented target for Lys^{48}- and Lys^{63}-linked polyubiquitination, but the effect of these modifications is not yet understood [84].

PTMs on DUBs Identified by Global Proteomics Studies

In addition to the biochemically-characterized examples of PTMs, several high-throughput studies aimed at mapping the phosphoproteome, ubiquitinome, and acetylome yielded information on additional post-translationally modified residues in DUBs (several such studies are summarized in Table 2, [65, 86–114]). Strikingly, large-scale phosphoproteomics studies have found 37 out of 55 USPs to be phosphorylated in vivo (reviewed in [115]). Global phosphoproteome analyses targeted to a particular kinase might be of special value, placing a phosphorylated DUB within a biological context. For instance, Matsuoka et al. [90] detected various DUBs as kinase substrates of ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3-related) in response to the DNA damage, which include USP1, UCHL3, USP19, USP24, USP28, and USP34, although the relevance of ATM/ATR-mediated phosphorylation of these enzymes is presently unclear [90]. Furthermore, proteomic studies such as [8] provide information on novel protein–protein interactions, including association with kinases, methyl transferases, and other proteins that might post-translationally modify DUBs.

All this indicates that the number of the PTMs affecting DUBs must be extensive, providing a great scope for future studies exploring roles of these already discovered modifications. Location of the modifiable residues within various DUB domains might give an initial clue on the mechanistic effect of PTMs on DUB function. For instance, different outcomes are to be expected for modifications occurring within the ubiquitin-binding domain, components of the catalytic site, or protein–protein interaction domains.

Table 2 List of all known post-translational modifications (PTMs) of deubiquitinating enzymes (DUBs)

Accession number	Entry name	Protein name	Length	Phosphorylation (residues)	References	Acetylation (residues)	References	Ubiquitination/ sumoylation (residues)	References
Q92560	BAP1_HUMAN	Ubiquitin carboxyl-terminal hydrolase BAP1	729	Ser327, Ser395, Thr487, Ser489, Ser582, Ser583, Ser592, Ser597	[65, 86, 87, 90]				
Q9NQC7	CYLD_HUMAN	Ubiquitin carboxyl-terminal hydrolase CYLD	956	Ser399, Ser418	[34, 35, 88]				
P46736	BRCC3_HUMAN	Lys-63-specific deubiquitinase BRCC36	316						
Q7RTX8	HIN1L_HUMAN	Putative HIN1-like protein	443						
Q5VVQ6	OTU1_HUMAN	Ubiquitin thioesterase OTU1 (OTU domain-containing protein 2) (DUBA-8)	348						
Q7L8S5	OTU6A_HUMAN	OTU domain-containing protein 6A (DUBA-2)	288						
Q8N6M0	OTU6B_HUMAN	OTU domain-containing protein 6B (DUBA-5)	293	Tyr272	[91]	Met1	[86]		
Q8TE49	OTU7A_HUMAN	OTU domain-containing protein 7A (Zinc finger protein Cezanne 2)	926						
Q6GQQ9	OTU7B_HUMAN	OTU domain-containing protein 7B (zinc finger protein cezanne) (zinc finger A20 domain-containing protein 1)	843	Ser100, Ser449, Ser464, Ser467	[65, 86, 87, 92, 93]				
Q96FW1	OTUB1_HUMAN	Ubiquitin thioesterase OTUB1 (otubain-1)	271	Ser16, Ser18, Tyr26	[50, 92]	Ala2, Lys188	[86, 114]		
Q96DC9	OTUB2_HUMAN	Ubiquitin thioesterase OTUB2 (otubain-2)	234						
Q5VV17	OTUD1_HUMAN	OTU domain-containing protein 1 (DUBA-7)	481						
Q5T2D3	OTUD3_HUMAN	OTU domain-containing protein 3	398	Ser224	[92]				
Q01804	OTUD4_HUMAN	OTU domain-containing protein 4 (HIV-1-induced protein HIN-1)	1113	Tyr438, Ser442, Ser556, Ser940, Ser1005, Ser1022, Ser1023	[86, 88, 89, 92–95]	Met1	[86]		
Q96G74	OTUD5_HUMAN	OTU domain-containing protein 5 (deubiquitinating enzyme A) (DUBA)	571	Ser64, Ser165, Tyr175, Ser177, Ser452, Thr507, Ser508	[86, 92, 93, 96, 97]				

Table 2 continued

Accession number	Entry name	Protein name	Length	Phosphorylation (residues)	References	Acetylation (residues)	References	Ubiquitination/ sumoylation (residues)	References
Q504Q3	PAN2_HUMAN	PAB-dependent poly(A)-specific ribonuclease subunit 2 (hPan2) (inactive ubiquitin carboxyl-terminal hydrolase 52)	1202	Ser791, Ser1189	[86, 89, 92]				
Q53GS9	SNUT2_HUMAN	U4/U6.U5 tri-snRNP-associated protein 2 (U4/U6.U5 tri-snRNP-associated 65 kDa protein) (65 K) (inactive ubiquitin-specific peptidase 39)	565	Ser42, Ser46, Ser82	[88, 92, 98]	Lys428	[114]		
P21580	TNAP3_HUMAN	Tumor necrosis factor alpha-induced protein 3 (TNF alpha-induced protein 3) (OTU domain-containing protein 7C) (putative DNA-binding protein A20) (zinc finger protein A20)	790	Ser459, Ser575, Ser381	[41, 88, 93]				
Q7RTZ2	U17L1_HUMAN	Putative ubiquitin carboxyl-terminal hydrolase 17-like protein 1	530						
Q6R6M4	U17L2_HUMAN	Ubiquitin carboxyl-terminal hydrolase 17-like protein 2 (deubiquitinating protein 3) (DUB-3)	530						
A6NCW0	U17L3_HUMAN	Ubiquitin carboxyl-terminal hydrolase 17-like protein 3	530						
A6NCW7	U17L4_HUMAN	Inactive ubiquitin carboxyl-terminal hydrolase 17-like protein 4	530						
A8MUK1	U17L5_HUMAN	Ubiquitin carboxyl-terminal hydrolase 17-like protein 5	530						
Q6QN14	U17L6_HUMAN	Ubiquitin carboxyl-terminal hydrolase 17-like protein 6	398						
P0C7H9	U17L7_HUMAN	Inactive ubiquitin carboxyl-terminal hydrolase 17-like protein 7	530						
P0C7I0	U17L8_HUMAN	Inactive ubiquitin carboxyl-terminal hydrolase 17-like protein 8	530						

Table 2 continued

Accession number	Entry name	Protein name	Length	Phosphorylation (residues)	References	Acetylation (residues)	References	Ubiquitination/ sumoylation (residues)	References
Q96FJ0	STALP_HUMAN	AMSH-like protease (AMSH-LP) (STAM-binding protein-like 1)	436	Ser25, Ser242	[92]				
Q14694	UBP10_HUMAN	Ubiquitin carboxyl-terminal hydrolase 10	798	Thr42, Thr100, Thr208, Ser211, Ser220, Ser226, Ser337, Ser364, Ser365, Ser370, Ser547, Ser563, Ser576	[65, 76, 86, 88, 89, 92, 93, 98–102]				
P51784	UBP11_HUMAN	Ubiquitin carboxyl-terminal hydrolase 11	963	Ser948, Ser953	[92]	Lys245	[114]		
O75317	UBP12_HUMAN	Ubiquitin carboxyl-terminal hydrolase 12	370						
Q92995	UBP13_HUMAN	Ubiquitin carboxyl-terminal hydrolase 13 (isopeptidase T-3) (ISOT-3)	863	Ser114, Thr122	[92, 93]				
P54578	UBP14_HUMAN	Ubiquitin carboxyl-terminal hydrolase 14	494	Tyr136, Ser143	[92, 93, 103]	Lys291, Lys313, Lys449	[114]		
Q9Y4E8	UBP15_HUMAN	Ubiquitin carboxyl-terminal hydrolase 15	981	Ser229, Ser961, Ser965	[86, 89, 92, 93]	Ala2	[86]		
Q9Y5T5	UBP16_HUMAN	Ubiquitin carboxyl-terminal hydrolase 16	823	Ser415, Ser552, Thr554	[65, 86, 92, 93]				
Q0WX57	UBP17_HUMAN	Ubiquitin carboxyl-terminal hydrolase 17	530						
Q9UMW8	UBP18_HUMAN	Ubl carboxyl-terminal hydrolase 18 (ISG15-specific-processing protease)	372						
O94966	UBP19_HUMAN	Ubiquitin carboxyl-terminal hydrolase 19	1318	Ser244, Ser1242	[65, 86, 90]				
O94782	UBP1_HUMAN	Ubiquitin carboxyl-terminal hydrolase 1	785	Ser13, Ser42, Ser67, Ser313, Ser475	[86, 90, 92, 96]				
Q9Y2K6	UBP20_HUMAN	Ubiquitin carboxyl-terminal hydrolase 20	914	Ser132, Ser134, Thr258, Ser263, Ser368, Ser373, Thr377, Ser406, Ser407, Ser413	[88, 92, 93, 97]				
Q9UK80	UBP21_HUMAN	Ubiquitin carboxyl-terminal hydrolase 21	565						
Q9UPT9	UBP22_HUMAN	Ubiquitin carboxyl-terminal hydrolase 22	525			Lys129	[114]		

Table 2 continued

Accession number	Entry name	Protein name	Length	Phosphorylation (residues)	References	Acetylation (residues)	References	Ubiquitination/ sumoylation (residues)	References
Q9UPU5	UBP24_HUMAN	Ubiquitin carboxyl-terminal hydrolase 24	2620	Ser1141, Ser1616, Ser1620, Ser1943, Tyr2024, Ser2047, Ser2077, Thr2559, Ser2561, Thr2565, Ser2604	[65, 86–90, 92, 93, 97, 102, 104–107]				
Q9UHP3	UBP25_HUMAN	Ubiquitin carboxyl-terminal hydrolase 25	1055	Tyr740, Tyr916	[80, 96]			Lys99 (SUMO), Lys99 (Ub), Lys141 (SUMO)	[78, 81]
Q9BXU7	UBP26_HUMAN	Ubiquitin carboxyl-terminal hydrolase 26	913						
A6NNY8	UBP27_HUMAN	Ubiquitin carboxyl-terminal hydrolase 27	438						
Q96RU2	UBP28_HUMAN	Ubiquitin carboxyl-terminal hydrolase 28	1077	Ser67, Ser714	[83, 90]				
Q9HBJ7	UBP29_HUMAN	Ubiquitin carboxyl-terminal hydrolase 29	922						
O75604	UBP2_HUMAN	Ubiquitin carboxyl-terminal hydrolase 2	605						
Q70CQ3	UBP30_HUMAN	Ubiquitin carboxyl-terminal hydrolase 30	517						
Q70CQ4	UBP31_HUMAN	Ubiquitin carboxyl-terminal hydrolase 31	1352	Tyr428, Ser1052, Thr1056	[91, 98]				
Q8NFA0	UBP32_HUMAN	Ubiquitin carboxyl-terminal hydrolase 32	1604	Tyr1137, Ser1361, Ser1372, Ser1376,	[86, 88, 92, 93]				
Q8TEY7	UBP33_HUMAN	Ubiquitin carboxyl-terminal hydrolase 33	942	Ser439	[92]				
Q70CQ2	UBP34_HUMAN	Ubiquitin carboxyl-terminal hydrolase 34	3546	Ser352, Ser355, Ser649, Ser658, Ser1503, Ser2488, Ser3358, Ser3359, Thr3381, Ser3406	[90, 92, 93, 104]				
Q9P2H5	UBP35_HUMAN	Ubiquitin carboxyl-terminal hydrolase 35	1017	Ser612	[92]				
Q9P275	UBP36_HUMAN	Ubiquitin carboxyl-terminal hydrolase 36	1121	Ser464, Ser494, Ser513, Ser515, Ser546, Ser582, Ser613, Ser614, Thr653, Ser667, Thr680, Ser682, Ser713, Ser742, Tyr874, Ser952, Ser1048	[86, 88, 92, 93, 96, 97, 106, 108]				

Table 2 continued

Accession number	Entry name	Protein name	Length	Phosphorylation (residues)	References	Acetylation (residues)	References	Ubiquitination/ sumoylation (residues)	References
Q86T82	UBP37_HUMAN	Ubiquitin carboxyl-terminal hydrolase 37	979	Ser650, Ser652	[86, 88, 92, 104]				
Q8NB14	UBP38_HUMAN	Ubiquitin carboxyl-terminal hydrolase 38	1042						
Q9Y6I4	UBP3_HUMAN	Ubiquitin carboxyl-terminal hydrolase 3	520	Thr141	[104]	Met1	[86]		
Q9NVE5	UBP40_HUMAN	Ubiquitin carboxyl-terminal hydrolase 40	1235						
Q3LFD5	UBP41_HUMAN	Putative ubiquitin carboxyl-terminal hydrolase 41	358						
Q9H9J4	UBP42_HUMAN	Ubiquitin carboxyl-terminal hydrolase 42	1325	Ser754, Ser856, Tyr953, Ser1220, Ser1223, Ser1227	[65, 86, 88, 92, 93, 97]				
Q70EL4	UBP43_HUMAN	Ubiquitin carboxyl-terminal hydrolase 43	1123	Tyr835, Ser1041	[92, 95]				
Q9H0E7	UBP44_HUMAN	Ubiquitin carboxyl-terminal hydrolase 44	712						
Q70EL2	UBP45_HUMAN	Ubiquitin carboxyl-terminal hydrolase 45	814						
P62068	UBP46_HUMAN	Ubiquitin carboxyl-terminal hydrolase 46	366						
Q96K76	UBP47_HUMAN	Ubiquitin carboxyl-terminal hydrolase 47	1375	Ser832, Tyr836, Ser910, Ser1353	[65, 86, 89, 92, 93, 97, 107]	Lys122	[114]		
Q86UV5	UBP48_HUMAN	Ubiquitin carboxyl-terminal hydrolase 48	1035	Ser886, Ser887, Ser888, Thr890	[86, 88]	Lys856	[114]		
Q70CQ1	UBP49_HUMAN	Ubiquitin carboxyl-terminal hydrolase 49	688						
Q13107	UBP4_HUMAN	Ubiquitin carboxyl-terminal hydrolase 4	963						
Q70EL3	UBP50_HUMAN	Inactive ubiquitin carboxyl-terminal hydrolase 50	339						
Q70EK8	UBP53_HUMAN	Inactive ubiquitin carboxyl-terminal hydrolase 53	1073						
Q70EL1	UBP54_HUMAN	Inactive ubiquitin carboxyl-terminal hydrolase 54	1684						
P45974	UBP5_HUMAN	Ubiquitin carboxyl-terminal hydrolase 5 (isopeptidase T)	858	Thr623, Ser783	[89, 93, 96]	Ala2, Lys184	[86, 114]		

Table 2 continued

Accession number	Entry name	Protein name	Length	Phosphorylation (residues)	References	Acetylation (residues)	References	Ubiquitination/ sumoylation (residues)	References
P35125	UBP6_HUMAN	Ubiquitin carboxyl-terminal hydrolase 6	1406						
Q93009	UBP7_HUMAN	Ubiquitin carboxyl-terminal hydrolase 7	1102	Ser18, Ser49, Thr54, Ser963	[65, 86, 92, 93]	Lys595, Lys869, Lys1084, Lys1096	[114]	Lys869 (Ub)	[66]
P40818	UBP8_HUMAN	Ubiquitin carboxyl-terminal hydrolase 8 (ubiquitin isopeptidase Y) (UBPy)	1118	Ser434, Ser452, Ser680, Ser718, Ser719, Thr907	[72, 74, 75, 86, 92, 93, 96]				
P09936	UCHL1_HUMAN	Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCH-L1)	223			Met1	[86]	Lys4 (Ub), Lys65 (Ub), Lys71 (Ub), Lys157 (Ub)	[57]
P15374	UCHL3_HUMAN	Ubiquitin carboxyl-terminal hydrolase isozyme L3 (UCH-L3)	230	Ser75, Ser130	[90, 92, 93]				
Q9Y5K5	UCHL5_HUMAN	Ubiquitin carboxyl-terminal hydrolase isozyme L5 (UCH-L5) (ubiquitin *C*-terminal hydrolase UCH37)	329			Lys158	[114]		
Q92738	US6NL_HUMAN	USP6 *N*-terminal-like protein (related to the *N*-terminus of tre)	828	Ser391, Ser396, Tyr582, Ser585, Ser617, Ser680, Tyr710, Ser716, Tyr729	[86, 88, 90, 92, 93, 105]				
Q93008	USP9X_HUMAN	Probable ubiquitin carboxyl-terminal hydrolase FAF-X (fat facets protein-related, X-linked) (fat facets in mammals) (hFAM)	2547	Thr583, Ser1593, Ser2436, Tyr2533, Ser2540	[86, 88, 89, 92–94, 97, 100]				
O00507	USP9Y_HUMAN	Probable ubiquitin carboxyl-terminal hydrolase FAF-Y (fat facets protein-related, Y-linked) (ubiquitin-specific protease 9, Y chromosome)	2555						
Q5W0Q7	USPL1_HUMAN	Ubiquitin-specific peptidase-like protein 1	1092						
Q96JH7	VCIP1_HUMAN	Deubiquitinating protein VCIP135 (valosin-containing protein p97/p47 complex-interacting protein p135)	1222	Ser747, Ser757, Thr761, Thr763, Tyr767, Thr770, Ser994, Ser998, Ser1198	[65, 86, 92, 93]	Lys408	[114]	Lys870 (Ub)	[110]

Table 2 continued

Accession number	Entry name	Protein name	Length	Phosphorylation (residues)	References	Acetylation (residues)	References	Ubiquitination/ sumoylation (residues)	References
Q8TAF3	WDR48_HUMAN	WD repeat-containing protein 48 (WD repeat endosomal protein) (USP1-associated factor 1) (p80)	677			Lys121, Lys214, Lys578	[114]		
Q9UGI0	ZRAN1_HUMAN	Ubiquitin thioesterase ZRANB1 (zinc finger Ran-binding domain-containing protein 1) (hTrabid)	708			Lys260	[114]		
P54252	ATX3_HUMAN	Ataxin-3 (Machado-Joseph disease protein 1) (spinocerebellar ataxia type 3 protein)	364	Ser340, Ser352	[46]				
Q8N594	MPND_HUMAN	MPN domain-containing protein	471	Ser178, Ser181	[88]				
Q5VVJ2	MYSM1_HUMAN	Histone H2A deubiquitinase MYSM1 (2A-DUB)	828	Ser218, Ser234, Thr236, Ser267	[90, 92, 93]				
O00487	PSDE_HUMAN	26S proteasome non-ATPase regulatory subunit 14	310	Tyr32, Ser150, Ser224	[94, 100, 109]				

The large portion of the data consists of PTMs detected in the global proteomics analyses, but it also includes modifications detected in targeted studies summarized in Table 1 (source: http://www.uniprot.org/ and listed references)

Ub ubiquitin, *SUMO* small ubiquitin-related modifier

Multi-PTM Crosstalk

Although there are multiple examples of post-translationally modified DUBs, the biochemical data is too scarce to draw any general conclusions, especially in relation to PTM-mediated regulation of the catalytic activity of DUBs. Future studies are likely to reveal trans-regulatory mechanisms of PTMs in the control of DUB catalytic activity and function. Such complex crosstalks between pathways have been recognized for many proteins, perhaps best described for kinases and histones. For instance, in some cases priming phosphorylation events are necessary to enable subsequent phosphorylation, sumoylation, or ubiquitination, while methylation or ubiquitination of certain residues in histones might be a prerequisite for their acetylation (reviewed in [116]). So far, no example of a similar mechanism has been discovered for DUBs, but they are anticipated. In particular, an occurrence of a phosphodegron, or a priming phosphorylation event necessary for recognition by an E3 ubiquitin ligase, leading to ubiquitination and proteasomal degradation, should be carefully examined for DUBs down-regulated by phosphorylation events. For instance, phosphorylation of USP25 [80] might trigger subsequent Lys^{48}-polyubiquitination resulting in proteasomal degradation. On the other hand, phosphorylation-driven negative regulation of ubiquitination might also be common. For example, it would be interesting to investigate this mechanism for USP8, since phosphorylation of Thr^{907} leads to accumulation of this protein [74, 75]. Another attractive aspect of post-translational events is a direct competition for a modifiable residue, such as for USP25, where Lys^{99} has been shown to be both ubiquitinated and sumoylated, with a potentially opposite functional outcome [78, 81].

Auto-Regulatory Mechanisms Keep DUBs in Check

Internal adaptive mechanisms controlling kinase enzymatic activity and therefore cell homeostasis have been known for a long time (reviewed in [117, 118]), but they have also been described for E3 ubiquitin ligases (e.g., Smurf2 [119]) and acetyltransferases (e.g., Rtt109 [120]). Since attachment of ubiquitin or ubiquitin-like molecules to protein substrates has been recognized as a multi-purpose regulatory modification, self-deubiquitination represents an attractive means of auto-regulation, whether it concerns control over lifespan, localization, or catalytic activity of DUBs. Indeed, this principle has been proposed for UCH-L1 [57], USP6 [59], and USP25 [78]. Monoubiquitination is particularly interesting since it impairs deubiquitinating properties of UCH-L1, while USP25 catalytic activity is most likely induced by this PTM [57, 78]. These studies indicate that auto-deubiquitination might contribute to both, inhibition and activation of the DUB function.

Further knowledge on how DUB function is regulated by PTMs may provide novel insights into their biology. Moreover, since many DUBs are implicated in cancer, inflammation, microbial disease, and neurodegeneration, novel insights into PTM-mediated regulation of DUBs might provide opportunities for combining inhibitors of DUBs and enzymes responsible for regulatory PTMs (e.g., kinase or phosphatase inhibitors) as more efficient entry points for pharmacological intervention strategies.

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