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

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 AMSSH 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 Lys63-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 Ser418. 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 Ser418 is not affected (Fig. 1a; [34]). There is some initial evidence that IKKγ (I kappa B kinase gamma) mediates CYLD phosphorylation on Ser418 [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 IKKβ (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 Lys63-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 Lys48-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 IKKβ kinase. Mass spectrometric analysis mapped the phosphorylation site to Ser318 that was verified in vitro and in vivo. IKKβ-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 Ser318 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].
| 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 IκB 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      | 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, 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]      |
| USP54      | Phosphorylation | Lys48- and Lys63-polyubiquitination |                |                  | Phosphorylation during mitosis                             | [85]       |
| USP16      | Phosphorylation |                |                         |                  | 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 | Akt-mediated | Possibly increased protein stability                      | [73]       |
| USP8       | Phosphorylation | Thr907         |                         |                  | Phosphorylated in response to ionizing irradiation         | [74, 75]   |

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.
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 Lys63-linked polyubiquitin chains, displaying even higher activity toward Lys63-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 Lys48-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 Ser16, Ser18, and Tyr26 [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. Ub-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 mono-ubiquitin 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 auto-regulatory 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 poly-ubiquitinated, 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 14-3-3e 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 exhibits 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 Thr42 and Ser337, and this event is required for the stabilization of USP10 and its translocation into nucleus after DNA damage. The alanine mutation of the Thr42/Ser337 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 Tyr740 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 Lys99 and Lys141, 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 Lys99. Mutation of this residue negatively regulates USP25-mediated stabilization of MyBPC1 and a mutually exclusive modification on Lys99—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 SCFbw7 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 Ser67 and Ser714 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 Lys48- and Lys63-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.
| Accession number | Entry name  | Protein name | Length (residues) | 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) | 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] |                        |            |                                       |            |
| Accession number | Entry name   | Protein name                                                                 | Length (residues) | 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               |                             |            |                       |            |                                      |            |
| P0C7L0           | U17L8_HUMAN  | Inactive ubiquitin carboxyl-terminal hydrolase 17-like protein 8                  | 530               |                             |            |                       |            |                                      |            |
| 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,    | [65, 76, 86, 88, 89, 92, 93, 98–102] |          |                        |           |                                      |           |
|                  |                    |                                                                               |        | Ser211, Ser220, Ser226,   |           |                        |           |                                      |           |
|                  |                    |                                                                               |        | Ser337, Ser364, Ser365,   |           |                        |           |                                      |           |
|                  |                    |                                                                               |        | Ser370, Ser547, Ser563,   |           |                        |           |                                      |           |
|                  |                    |                                                                               |        | Ser576                    |           |                        |           |                                      |           |
| 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]      |                                      |           |
| Q9YST5           | 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    | Ser13, Ser134, Thr258,    | [88, 92, 93, 97] |                        |           |                                      |           |
|                  |                    |                                                                               |        | Ser263, Ser368, Ser373,    |           |                        |           |                                      |           |
|                  |                    |                                                                               |        | Thr377, Ser406, Ser407,    |           |                        |           |                                      |           |
|                  |                    |                                                                               |        | Ser413                    |           |                        |           |                                      |           |
| Q9UK80           | UBP21_HUMAN        | Ubiquitin carboxyl-terminal hydrolase 21                                      | 565    |                           |           |                        |           |                                      |           |
| Q9UPT9           | UBP22_HUMAN        | Ubiquitin carboxyl-terminal hydrolase 22                                      | 525    |                           |           | Lys129                 | [114]     |                                      |           |
| Accession number | Entry name | Protein name                          | Length (residues) | 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] |                        |            |                                      |            |
| 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   |                           |            |                        |            |                                      |            |
| Q9Y6l4           | UBP3_HUMAN       | Ubiquitin carboxyl-terminal hydrolase 3          | 520    | Thr141                    | [104]      | Met1                   | [86]       |                                      |            |
| Q9NVE5           | UBP40_HUMAN      | Ubiquitin carboxyl-terminal hydrolase 40         | 1235   |                           |            |                        |            |                                      |            |
| Q3LFDS           | UBP41_HUMAN      | Putative ubiquitin carboxyl-terminal hydrolase 41| 358    |                           |            |                        |            |                                      |            |
| Q9H9l4           | 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] |                                      |            |
| Accession number | Entry name       | Protein name                                      | Length (residues) | Phosphorylation (residues) | References | Acetylation (residues) | References | Ubiquitination/sumoylation (residues) | References |
|------------------|------------------|--------------------------------------------------|-------------------|---------------------------|------------|------------------------|------------|--------------------------------------|------------|
| P35125           | UBP6_HUMAN       | Ubiquitin carboxyl-terminal hydrolase 6          | 1406              | Ser18, Ser49, Thr54, Ser963 | [65, 86, 92, 93] | Lys595, Lys869, Lys1084, Lys1096 | [114]      | Lys869 (Ub)                          | [66]       |
| 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] | Lys869 (Ub)                          | [66]       |
| P09936           | UCHL1_HUMAN      | Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCH-L1) | 223               | Met1                       | [86]       | Lys4 (Ub), Lys869 (Ub), Lys1084, Lys1096 | [57]       |
| P15374           | UCHL3_HUMAN      | Ubiquitin carboxyl-terminal hydrolase isozyme L3 (UCH-L3) | 230               | Ser75, Ser130              | [90, 92, 93] | Lys869 (Ub)                          | [66]       |
| Q9Y5K5           | UCHL5_HUMAN      | Ubiquitin carboxyl-terminal hydrolase isozyme L5 (UCH-L5) (ubiquitin C-terminal hydrolase UCH37) | 329               | Lys158                     | [114]      | Lys869 (Ub)                          | [66]       |
| 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] | Lys869 (Ub)                          | [66]       |
| 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] | Lys869 (Ub)                          | [66]       |
| O00507           | USP9Y_HUMAN      | Probable ubiquitin carboxyl-terminal hydrolase FAF-Y (fat facets protein-related, Y-linked) (ubiquitin-specific protease 9, Y chromosome) | 2555              |                          |            | Lys869 (Ub)                          | [66]       |
| Q5W0Q7           | USPL1_HUMAN      | Ubiquitin-specific peptidase-like protein 1      | 1092              |                          |            | Lys869 (Ub)                          | [66]       |
| Q96JH7           | VCIP1_HUMAN      | Deubiquitinating protein VCIP135 (valosin-containing protein p97/p47 complex-interacting protein p135) | 1222              | Ser747, Ser757, Thr761, Thr763, Tyr767, Thr770, Ser994, Ser998, Ser198 | [65, 86, 92, 93] | Lys408                               | [114]      | Lys870 (Ub)                          | [110]      |
| Accession number | Entry name       | Protein name                                                                 | Length (residues) | 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 Lys48-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 Thr907 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 Lys99 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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