Mechanisms orchestrating the enzymatic activity and cellular functions of deubiquitinases

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Deubiquitinases (DUBs) are required for the reverse reaction of ubiquitination and act as major regulators of ubiquitin signaling processes. Emerging evidence suggests that these enzymes are regulated at multiple levels in order to ensure proper and timely substrate targeting and to prevent the adverse consequences of promiscuous deubiquitination. The importance of DUB regulation is highlighted by disease-associated mutations that inhibit or activate DUBs, deregulating their ability to coordinate cellular processes. Here, we describe the diverse mechanisms governing protein stability, enzymatic activity, and function of DUBs. In particular, we outline how DUBs are regulated by their protein domains and interacting partners. Intramolecular interactions can promote protein stability of DUBs, influence their subcellular localization, and/or modulate their enzymatic activity. Remarkably, these intramolecular interactions can induce self-deubiquitination to counteract DUB ubiquitination by cognate E3 ubiquitin ligases. In addition to intramolecular interactions, DUBs can also oligomerize and interact with a wide variety of cellular proteins, thereby forming obligate or facultative complexes that regulate their enzymatic activity and function. The importance of signaling and post-translational modifications in the integrated control of DUB function will also be discussed. While several DUBs are described with respect to the multiple layers of their regulation, the tumor suppressor BAP1 will be outlined as a model enzyme whose localization, stability, enzymatic activity, and substrate recognition are highly orchestrated by interacting partners and post-translational modifications.

The attachment of ubiquitin (Ub) moieties to proteins is a highly conserved post-translational modification in eukaryotes. Protein ubiquitination is catalyzed by E1 Ub-activating, E2 Ub-conjugating, and E3 Ub-ligating enzymes, culminating in the modification of internal lysines or N-terminal residues of proteins (1–4). Ligation of Ub to proteins regulates different signaling pathways and cellular processes by inducing changes in protein function or targeting proteins for proteasomal degradation (4–9). Deubiquitinases (DUBs) constitute a superfamily of proteases that participate in the timely reversal of protein ubiquitination, thus controlling the functional outcomes of this post-translational modification (10–15). In mammals, DUBs can be classified into seven major families based on sequence conservation of the catalytic domain (Fig. 1). These include the Ub carboxy-terminal hydrolases (UCH), the Ub-specific proteases (USP), the Machado–Josephin domain-containing proteases, the ovarian tumor proteases (OTU), the JAMM/MPN+ metalloproteases (JAMM), the motif interacting with Ub–containing novel DUB family, and the recently discovered Zinc finger-containing Ub peptidase 1 (12). Most DUB enzymes are cysteine proteases, which are characterized by a catalytic triad containing, notably, cysteine and histidine residues. Their mechanism of catalysis involves a nucleophilic attack mediated by the cysteine thiol side chain, which results in the cleavage of the peptide bonds. On the other hand, the JAMM/MPN+ family of DUBs are metalloproteases that use a zinc atom coordinated by histidine and aspartic acid to ensure catalysis and Ub removal (10–15).

In addition to representing a substantial class of proteins in higher eukaryotes, DUBs are also found in viruses, bacteria, and yeast (12, 16–28). Studies in eukaryotes indicate that DUBs regulate a wide spectrum of cellular processes including protein quality control, membrane receptor signaling, endocytosis, DNA-dependent processes, cell cycle regulation, differentiation, cell survival, and cell death (10–15). Moreover, these enzymes have emerged as key factors in the cellular responses that orchestrate host–pathogen interactions (16–30).

Several mechanisms of control ensure the spatiotemporal deubiquitination of substrates and prevent unrestrained DUB catalysis. This includes mechanisms influencing gene expression levels, protein abundance, folding, and tissue distribution (10–15). Moreover, DUBs contain a variety of domains and motifs that could be post-translationally modified to regulate their subcellular localization, conformation, protein–protein interaction, and enzymatic activity (10–15).

In this review, we outline regulatory mechanisms responsible for controlling DUB stability, localization, and enzymatic

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activity as well as coordinating protein interactions and multi-protein complex assembly. While we provide examples of regulation for key DUBs in multiple families, the tumor suppressor BAP1 is outlined as a model DUB that is subjected to multiple levels of tight regulation. BAP1 is an essential DUB best known for its DUB activity toward histone H2AK119ub and the regulation of chromatin-associated processes (9, 31, 32). BAP1 is mutated in multiple cancers including malignant pleural mesothelioma, uveal melanoma, renal cell carcinoma, and intrahepatic cholangiocarcinoma, rendering this enzyme as the most frequently mutated DUB in human cancers (32, 33).

Regulation of DUBs by intramolecular interactions and self-assembly

**Intramolecular interactions and DUB catalysis**

Most DUBs have modular structures and contain diverse domains, nonorganized extensions and motifs, in addition to their catalytic domains. These additional structures can engage in intramolecular and intermolecular interactions and play critical roles in coordinating DUB activity and function (12, 13, 34–36). Moreover, several DUBs contain one or multiple Ub-binding domains as well as insertions of variable lengths inside their catalytic domains (12, 34, 35). The importance of intramolecular interactions is notably provided by the Ub specific protease 7 (USP7) (also termed Herpes-Associated Ub-Specific Protease (HAUSP)), which contains a TNF receptor-associated factor (TRAF) domain, a catalytic domain in the N-terminal region as well as five Ub-like domains (UBLs) in the C-terminal region (Fig. 2A). As a member of the USP family, USP7 is characterized by architectural features known as the palm, the thumb, and the finger subdomains. While the finger subdomain is mostly associated with Ub-binding, the catalytic triad responsible for hydrolysis is localized at the junction between the palm and the thumb subdomains (35). To enable DUB enzymatic activity, USP7 uses its most distant C-terminal UBL domain to stabilize and coordinate its catalytic site (37–39) (Fig. 2B). To allow USP7 to switch between active and inactive conformations, this DUB partly relies on a long α-helix, termed the connector helix, that connects the catalytic domain to the first UBL domain (40). Furthermore, linker regions separating the UBL domains permit intramolecular flexibility and rearrangements. Indeed, the binding of Ub to USP7 promotes the localization of the C-terminal tail within an activation cleft located within the catalytic domain of USP7 (37–39) (Fig. 2B). To allow USP7 to switch between active and inactive conformations, this DUB partly relies on a long α-helix, termed the connector helix, that connects the catalytic domain to the first UBL domain (40). Indeed, GMP-synthetase
interacts with UBL1-2-3 and stabilizes UBL4-5 at the catalytic domain of USP7 (38). Altogether, these results suggest that USP7-mediated deubiquitination is achieved by intramolecular interactions. The multiple domains of USP7 endow this DUB with the functional versatility to deubiquitinate many substrates and regulate various cellular processes including DNA damage signaling, epigenetic control of gene expression, viral infection, and immune response (42, 44).

Figure 2. DUB regulation by intramolecular interactions and self-assembly. A, schematic representation of USP7 domain organization and boundaries. USP7 catalytic triad is shown in red as well as the C-terminal tail (K1084 to N1102) required for its activation in black. B, USP7 self-activation. USP7 adopts two conformations respectively associated with an activated and an inactivated state. The switch from the inactive to the active conformation requires USP7 C-terminal tail binding into an activating cavity located in the catalytic domain (CD). This leads to a conformational rearrangement of the UBLs that takes place in the presence of the ubiquitin-conjugated substrate. This rearrangement might also involve a long flexible charged α-helix positioned at the interface between the CD and the UBLs. Once the C-terminal tail is engaged into the activation cleft, the catalytic domain is stabilized and fully active. The “plus” signs show the charged helix. C, schematic representation of Calypso and ASX domain organization and boundaries. Calypso catalytic triad is shown in red. D, Calypso dimerization promotes its recruitment to the nucleosomes. The Drosophila Calypso and ASX proteins form a 2:2 stoichiometric complex. This assembly is needed for chromatin recruitment and catalytic activity toward H2AK118ub. ASX, additional-sex comb; CC1/2, coiled-coil 1/2; CD, catalytic domain; CTE, C-terminal extension, DEUBAD, DEUbiquitinase ADaptator; NLS, nuclear localization signal; NTE, N-terminal extension; PHD, plant homeo-domain; TRAF, tumor necrosis factor receptor–associated factor; Ub, ubiquitin; UBL, ubiquitin-like; UCH, ubiquitin carboxy-terminal hydrolase; ULD, UCHL5-like domain.
Intramolecular interactions between distinct DUB domains can also be permanent, as part of the 3D structure of the enzyme. An example of a stable intramolecular interaction is provided by the UCH family DUB BAP1 (45–49). BAP1 is localized predominantly in the nucleus, as part of a large multiprotein complex (discussed in section 3). BAP1 and its Drosophila ortholog Calypso contain a highly conserved UCH catalytic domain adjacent to a small coiled-coil motif, followed by an insertion in the middle of the protein (termed the nonorganized regions for BAP1), and then a C-terminal domain (CTD) containing a coiled-coil motif adjacent to the nuclear localization signal (NLS) (Fig. 2C). Through coiled-coil motif interactions, the catalytic domain of BAP1/Calypso establishes a stable interaction with the CTD. The UCH–CTD interaction is important for the stimulation of BAP1/Calypso DUB activity by cofactors (45–49) (Fig. 2D). A similar strategy of 3D organization is employed by UCHL5 (UCH37), a component of the proteasome and the INO80 chromatin-remodeling complex (50–55). Of note, BAP1 and UCHL5 also use a similar mechanism of regulation by their respective cofactors, involving a distinct domain termed DEUBD (DEUBiquitinase ADaptor) found in ASXLs, ADRM1 (RPN13), and NFRKB (INO80G) (described in section 3). Overall, these studies assert the importance of intramolecular interactions between the catalytic domain and other DUB domains for the control of enzymatic activity and regulation by cofactors.

**DUB oligomerization and catalysis**

Another level of DUB regulation involves their oligomerization. For example, USP25 is assembled into a homotetrameric quaternary complex that inhibits its enzymatic activity (Fig. 3) (56–58). A coiled-coil insertion within the catalytic domain is extended by a disordered sequence that contacts the catalytic site in the tetramer, but not in the active dimer, precluding Ub binding (56–58). In support of this autoinhibitory mechanism, cancer-associated mutations that disrupt this intermolecular interaction lead to relief from autoinhibition, emphasizing the biological importance of USP25 oligomerization states (57). Currently, it remains unclear whether specific molecular signals, interacting partners, and/or post-translational modifications regulate the oligomerization states of USP25 and hence its enzymatic activity.

While the example of USP25 indicates how oligomerization induces DUB autoinhibition, self-assembly can also promote DUB activity. The Drosophila ortholog of BAP1, Calypso, was recently shown to undergo dimerization (48). While this assembly is not directly required for stimulating catalytic activity, it favors the recruitment of this DUB to chromatin, whereby it can access and deubiquitinate histone H2Aub. The dimerization of Calypso requires the coiled-coil regions that are also conserved in human BAP1. Interestingly, Calypso dimerization would simultaneously position the two UCH domains near the two-ubiquitination sites of the H2A dimer within the nucleosome (Fig. 2D). Thus, Calypso/BAP1 interaction with nucleosomes and subsequent deubiquitination of H2Aub appear to be highly coordinated. Nonetheless, further studies are needed to determine how BAP1 dimerization regulates its DUB activity in vivo. In particular, it will be worthwhile to define how transcription factors and chromatin-associated proteins cooperate with the BAP1 dimer to ensure timely deubiquitination of H2AK119ub at defined genomic regions.

In summary, DUBs can undergo oligomerization, regulating intrinsic DUB activity and access to substrates, thus providing an important level of regulation. The extent to which transient or stable oligomerization can be generalized to the majority of DUBs and whether this influences their functions remain to be determined.

**DUB action through diverse modes of association with E2-conjugating and E3 ligases**

**DUB-mediated inhibition of ubiquitination by diverting E2s from E3s**

DUBs do not necessarily act only following the action of E2 Ub-conjugating enzymes and E3 Ub ligases to terminate ubiquitination reactions (10–14). Indeed, DUBs can actively participate, in conjunction with E2 and E3 enzymes, to orchestrate Ub-signaling events. For instance, an intricate relationship between DUBs and E2s is exemplified by OTUB1, a DUB involved in DNA damage signaling and immune regulation. OTUB1 inhibits chromatin-associated ubiquitination events mediated by the E3 Ub ligase RNF168, which occur during the cellular response to DNA double-strand breaks (59). Mechanistically, OTUB1 interacts with and inhibits several E2 Ub-conjugating enzymes, including UBC13 and UBCH5. These events are mediated in a DUB catalytic activity–independent manner and result in the inhibition of Ub chain elongation. This occurs through binding of the DUB to E2–Ub thioester intermediates, thus diverting them from their cognate E3 Ub ligases (59–61). In addition, OTUB1–E2 interactions stimulate OTUB1-mediated cleavage of K48-linked Ub chains through conformational changes of this DUB. Interestingly, this is further modulated by E2 charging and free Ub, providing a potential mechanism for the coordination of signaling processes with Ub metabolism (60, 62).

DUBs also assemble into multiprotein complexes wherein they simultaneously interact with both E2 Ub-conjugating enzymes and E3 Ub ligases. For instance, the DUB Ataxin3 (AT3), whose polyglutamine expansion underlies the Machado–Joseph neurodegenerative disorder (63), interacts with UBC7 and UBCH7 E2 Ub-conjugating enzymes as well as the E3 Ub ligase Parkin. Parkin promotes autophagy-mediated clearance of damaged mitochondria, and its mutation or deregulation is also involved in the pathogenesis of Parkinson’s disease (64, 65). AT3 deubiquitinates Parkin and their interaction is strongly promoted by Parkin auto-ubiquitination. AT3 subsequently stabilizes an unproductive UBC7–Parkin complex that limits Parkin autoubiquitination, possibly through inhibition of the E2 release from Parkin and its subsequent charging by Ub. In addition, the E2-mediated transfer reaction might be diverted toward Ub ligation to AT3 itself (64, 65). While the significance of AT3 interactions with E2s/
DUB complexes: Composition, regulation, and functions

| Factors | Subunit organization and function | Complex structure |
|---------|----------------------------------|------------------|
| BRCA1-A complex (JAMM) / DNA repair | K63 poly-Ub chain DUB involved in several biological processes | Refs: 108, 109, 192, 193 |
| BRCC36 | Cofactor involved in BRCA1-A dimerization and BRCC36 DUB activity through its interaction with JAMM/MPN- domain | |
| ABRAAXS | Interacts with BRCA1-A and binds mixed SUMO/K63 Ub chains | |
| RAP80 | Interacts with MERIT40, mediates RAP80 interaction and BRCA1 sequestration, and binds Ub | |
| BRE | Mediates RAP80 integration and binds Ub | |
| MERIT40 | Promotes homologous recombination | |
| BRCC36 | K63 poly-Ub chain DUB involved in several biological processes | Refs: 102, 105, 108, 109 |
| ABRO1 | Cofactor involved in BRCC36 DUB activity through its interaction with JAMM/MPN- domain and recruits SHMT2 | |
| BRE | Involved in SHMT2 recruitment, interacts with ABRO1, MERIT40, and binds Ub | |
| SHMT2 | Blocks BRCC36 DUB activity through the exclusion of Ub chains from its catalytic site | |
| OTULIN-LUBAC complex (OTU) / Cell death, immunity | Met1 poly-Ub chain DUB. Stabilizes the LUBAC complex by promoting its poly-deubiquitination | Refs: 74, 80, 81, 83, 88, 88, 194 |
| OTULIN | Subunit of the LUBAC complex and a RBR (RING-Between-RING) E3 ligase that catalyzes Met1-Ub-mediated ubiquitination. It also interacts with OTULIN | |
| HOIP | Subunit of the LUBAC complex and a RBR E3 ligase that monoubiquitinitates all the subunits | |
| SHARPIN | Subunit of the LUBAC complex. SHARPIN is a scaffold subunit that stimulates HOIP activity and is required for LUBAC formation and stability | |
| CYLD-LUBAC complex (USP) / Cell death, immunity | Met1/K63 poly-Ub chain DUB | Refs: 74, 90, 91, 194, 195 |
| CYLD | Required for CYLD recruitment to its target substrates and stimulates its activity. Also acts as a bridging factor between CYLD and HOIP | |
| SPATA2 | Catalyzes Met1-Ub-mediated ubiquitination. Also acts as a scaffold through its interaction with SPATA2 | |
| HOIP | Subunit of the LUBAC complex and a RBR E3 ligase that monoubiquitinitates all the subunits | |
| SHARPIN | Subunit of the LUBAC complex. SHARPIN is a scaffold subunit that stimulates HOIP activity and is required for LUBAC formation and stability | |
Parkin remains incompletely understood, the above findings emphasize the intricate partnerships between Ub ligation and deubiquitination in fine-tuning Ub-signaling events.

Consistent with the variety of cellular processes in which DUBs limit the access of E3s to cognate E2s, the zinc finger DUB A20 (also known as tumor necrosis factor, alpha-induced protein 3) constitutes an important regulator of inflammation. A20 negatively regulates the nuclear factor kappa B (NF-κB) and inflammation through multiple mechanisms \(^{66–69}\), including the targeting of receptor-interacting protein 1 of the TNF receptor signaling pathway for proteasomal degradation through its own E3 Ub ligase activity \(^{67}\). A20 blocks the interactions between the E3 Ub ligases TRAF6, TRAF2, and cIAP1 and the corresponding E2 Ub-conjugating enzymes.
UBC13 and UBCH5C. This ensures the targeting of the latter enzymes for proteasomal degradation (69).

In summary, in addition to Ub removal from proteins, DUBs can also limit substrate ubiquitination by interfering with E2s and E3s, providing additional means to tightly regulate ubiquitin-signaling events.

DUBs modulating ubiquitination through direct interaction with E3s

A notable example of direct DUB-E3 coordination is provided by the LUBAC (linear Ub chain assembly complex) E3 Ub ligase, which is known to interact with two DUBs; OTULIN (OTU DUB with linear linkage specificity) and CYLD (cylindromatosis). LUBAC is an E3 Ub ligase complex composed of three interacting partners: SHARPIN (Shank-associated RH domain-interacting protein), HOIP (HOIL-1-interacting protein), and HOIL-1 (heme-oxidized IRP2 Ub ligase-1) (Fig. 3) (70–74). It functions as a linear Met1-Ub chain conjugating E3 complex that regulates innate immune signaling, notably by promoting NF-κB activation (72, 75–78). HOIP is activated through association with its interacting partners, which triggers the ligation of linear Ub chains on receptor-interacting protein 1 (RIPK1) and NEMO (IKKγ), two downstream activators of NF-κB signaling (72, 73, 77, 79).

Furthermore, HOIL-1 catalyzes the monoubiquitination of LUBAC subunits, which primes for HOIP-mediated linear Ub chain extension, therefore dampening LUBAC activity (80). Meanwhile, OTULIN specifically cleaves the Met1-Ub chains generated by LUBAC (81, 82). The PIM (PUB-interaction motif) domain of OTULIN interacts specifically with the PUB domain of HOIP to ensure DUB-E3 interaction (83, 84).
Regulation of DUBs through assembly into large heteromeric and multienzymatic complexes

**DUB assembly in mutually exclusive complexes**

Several DUBs are assembled into large stable multiprotein complexes found in several tissues and subcellular compartments (12, 13, 35) (Fig. 3). Indeed, a systematic purification and mass spectrometry identification of DUB complexes and interacting partners indicated that these enzymes are associated with a plethora of proteins and enzymes covering a wide spectrum of cellular functions and processes (93). Association into large complexes endows DUBs with additional interaction interfaces to regulate substrate recruitment and modulation of enzymatic specificity and activity. BRCC36 provides a prominent example of a DUB assembled in two distinct complexes in a mutually exclusive manner. BRCC36 is a member of the JAMM family DUBs that exhibit a preference for K63-linked Ub chains (94). BRCC36 is found in the BRCA1-A complex and the BRCC36 isopeptidase complex (BRISC), which are involved in DNA repair and immune signaling, respectively (95–106) (Fig. 3). Structural studies indicate that BRCC36 and ABRAXAS2 or ABRO1 form heterodimers, which ensure enzymatic activity and association with additional cofactors including RAP80 and BRCA1 (in the BRCA1-A complex) or the serine hydroxymethyltransferase 2 (SHMT2) (in the BRISC complex) (107–109). Of note, the dimeric form of SHMT2 interacts with the BRISC complex and blocks the catalytic site of BRCC36, thereby preventing deubiquitination. However, SHMT2 can also undergo tetramerization, which results in its dissociation from BRISC and the subsequent activation of BRCC36 (106). Thus, BRCC36 exemplifies how a DUB can be regulated by its interacting partners to mediate distinct cellular processes. Nonetheless, several questions await further studies to fully establish how BRCC36 is regulated. In particular, it remains unknown the manner by which BRCC36 molecules are allocated to nuclear (BRCA1-A) or cytoplasmic (BRISC) complexes. In addition, how BRCC36-mediated deubiquitination is coordinated with other functions of its associated complexes also remains incompletely understood.

Similar to BRCC36, UCHL5 is a component of two distinct multiprotein assemblies. Indeed, UCHL5 interacts, in a mutually exclusive manner, with the proteasome or the INO80 ATPase chromatin remodeling complex (Fig. 3) (51–54, 110, 111). UCHL5 uses its ULD (UCHL5-like domain) to interact with the DEUBAD domain of the proteasome subunit RPN13, promoting a conformational change that activates UCHL5 (53, 55). Notably, the active site cross-over loop, normally localized above the catalytic cysteine inside the Ub-binding site of UCHL5, is repositioned to allow deubiquitination (53, 55). Functionally, UCHL5 recruitment to the proteasome ensures poly-Ub chain debranching, enhancing proteasomal degradation, and promoting proper cell cycle progression (112, 113). Additionally, UCHL5 can also bind the DEUBAD of NFRKB, a subunit of the INO80 chromatin-remodeling complex (52, 53, 55). However, in contrast to RPN13, NFRKB inhibits UCHL5 DUB activity. A spatial rearrangement of an NFRKB loop with the ULD and UCH domains prevents substrate docking and deubiquitination (53, 55).

DUBs can also compete for specific partners. For instance, UAF1 (USP1-associated factor 1), first identified as a factor that activates USP1 DUB activity (114), was later shown to interact with and activates, in a mutually exclusive manner, several DUBs including USP12 and USP46 (Fig. 3) (115–119). UAF1 contains three domains with an N-terminal β-propeller domain, a central ancillary domain, and a C-terminal SUMO-like domain. The β-propeller domain of UAF1 binds to the distal end of the USP finger and promotes structural rearrangements in the Ub-binding site, increasing its overall activity. Adding more complexity, USP12 and USP46 display a
high degree of homology (88%) and can both interact with an additional coactivator named WDR20 (116, 119–122). Composed of multiple WD40-repeat motifs, WDR20 interacts with the back of the finger and the palm subdomains of USP12/46, which triggers conformational modifications in the catalytic center of these DUBs. These events enhance the activity of USP12 and USP46, independently of UAF1 (119, 120). Thus, UAF1 and WDR20 represent allosteric activators that mediate, through multiple structural rearrangements, the synergistic activation of USP12 and USP46. Of note, both UAF1 and WDR20 are subjected to multiple post-translational modifications, including phosphorylation and ubiquitination (PhosphoSite database (123)), but the significance of these modifications with respect to modulation of DUB activity remains to be established.

**DUB assembly in multiprotein complexes with distinct enzymatic activities**

DUBs can also be integrated into large multiprotein complexes with several subcomplex modules. This is the case for the highly conserved Spt-Ada-Gcn5 acetyltransferase (SAGA) transcription coactivator complex, which has multiple structural and functional modules including a core module, a histone acetyltransferase module and a DUB module (Fig. 3) (124). In yeast, the SAGA DUB module is composed of the DUB Ubp8 and three subunits, Sgf11, Sus1, and Sgf73, that are all required for deubiquitination of H2BK123 (K120 in mammals), a modification associated with gene transcription by RNA polymerase II (124–127). Indeed, the four subunits establish extensive contacts with each other, illustrating the importance of complex assembly for DUB activity (125, 126). In addition, the DUB module creates contacts with nucleosomes through the zinc finger domain of Sgf11 which interacts with the H2A/H2B acidic patch, while Ubp8 forms contacts with H2B and Ub (125, 126). Moreover, structural studies indicate that positioning of the multiple SAGA modules within the supercomplex ensure coordinated deubiquitination and acetylation of nucleosomes to promote gene transcription (125, 128–131). Thus, the SAGA multiprotein complex provides a notable example of how DUB enzymatic activity is tightly controlled by associated proteins. In addition, it also shows how a DUB, as being part of a multifunctional complex, can exert its function in concert with additional activities.

The DUB BAP1 assembles several multiprotein complexes containing diverse chromatin-associated factors (Fig. 3). These include the additional sex comb–like proteins (ASXL1/ASXL2/ASXL3), the host cell factor 1, the O-linked N-acetylglucosamine transferase, and the lysine demethylase KDM1B/LSD2 (93, 132, 133). While host cell factor 1, O-linked N-acetylglucosamine transferase, and KDM1B are known regulators of transcription and other chromatin-associated processes (134–141), their exact coordination with BAP1 DUB activity remains incompletely understood. Nonetheless, BAP1 might deubiquitinate and stabilize these cofactors or their associated proteins to regulate transcription (140, 141). BAP1 also associates with transcription factors, including FOXK1, FOXK2, and Yin Yang1 (YY1), which ensure the recruitment of this DUB to chromatin (133, 142). Of interest, BAP1 forms mutually exclusive complexes with ASXLs, which play important roles in maintaining BAP1 stability and promoting its DUB activity. Notably, similar to the RPN13-UCHL5 interaction, these factors also use their DEUBAD to interact with the CTD of BAP1 (see above). These interactions are necessary for histone H2AK119ub deubiquitination and transcription regulation by this DUB. The UCH and CTD domains of BAP1 as well as the DEUBAD form a composite Ub-binding interface that interacts with the hydrophobic and the charged patches of ubiquitin, thus ensuring catalysis (Fig. 4A) (46). Notably, cancer-associated mutations of BAP1 that inhibit its interaction with ASXLs also engender abrogation of its DUB activity toward H2AK119ub and reduce cell proliferation. How BAP1 activity is dynamically controlled by its partners in diverse ASXL complexes and contexts remains a fundamental question with respect to understanding the regulation of this tumor suppressor.

In summary, DUB function is intimately orchestrated by interacting partners that regulate protein stability or activity. Several DUBs are integrated into large complexes with multiple functions. However, how DUBs are regulated within such complexes in response to developmental, physiological, or stress-associated signaling remains an area of active investigation.

**Regulation of DUBs by ubiquitination and self-deubiquitination**

**Regulation of DUB catalytic activity by ubiquitination**

Several DUBs were shown to be regulated by ubiquitination targeting the catalytic domain or other regions of the enzyme. The biological significance and exact mechanisms controlling these ubiquitination events are far from being fully established. Nonetheless, over the years, valuable information has been gained regarding the regulation imposed by this post-translational modification on interactions and activity of specific DUBs. For instance, UCHL1 is monoubiquitinated in its catalytic domain, blocking its capacity to bind Ub and substrates (Fig. 5) (143). Importantly, mutation of residues that abolish Ub-binding by UCHL1 also inhibits its mono-ubiquitination. This intriguing dependency of DUB ubiquitination on Ub-binding suggests that there may exist an intermediate step of noncovalent Ub transfer occurring between the catalytic domain of UCHL1 and the E2-E3 complex. Additionally, mutation of the catalytic cysteine results in increased monoubiquitination of UCHL1, suggesting that its ubiquitination state is reversed by self-deubiquitination through intramolecular interactions. Thus, it is possible that the equilibrium between ubiquitination and deubiquitination regulates UCHL1 DUB activity to ensure a tight control of its activity toward substrates (143).

The DUB AT3 described above (section 2A) is also associated with ubiquitination-dependent quality control mechanisms (Fig. 5) (144–146). AT3 is constitutively ubiquitinated near the catalytic site, and this ubiquitination state is increased
following proteasome inhibition or when the unfolded protein response is induced by treatment with dithiothreitol. The ubiquitinated form of AT3 is more effective in cleaving Ub chains, indicating that Ub modification increases AT3 catalytic activity (147, 148). AT3 ubiquitination might thus constitute a feedback mechanism that links Ub metabolism to DUB activity. However, AT3 regulation appears to be more complex, as the catalytic activity of this DUB also regulates its own stability and cellular localization (149, 150). While the significance of these events remains unclear, they suggest that AT3 ubiquitination plays an important role in controlling its activity and stability.

**Regulation of DUB localization by ubiquitination**

Self-deubiquitination of BAP1 provides a notable example of DUB regulation at the level of subcellular localization (Fig. 4B). The E2-conjugating/E3-ligase hybrid UBE2O is a substoichiometric component of BAP1 complexes (93, 132, 133). UBE2O monoubiquitinates multiple lysines on the BAP1 NLS, thereby inducing its cytoplasmic sequestration (45). These ubiquitination events are actively counteracted by BAP1 self-deubiquitination which, importantly, could only be observed when the BAP1 UCH domain interacts with the CTD (Fig. 4B). Indeed, the UCH–CTD interaction brings the NLS of BAP1 close enough to the catalytic domain to promote self-deubiquitination (45). Thus, UBE2O-mediated ubiquitination of BAP1 might constitute a quality control mechanism that prevents the nuclear import of an improperly folded DUB. Consistent with this notion, it was later found that UBE2O targets orphan proteins that fail to assemble into their cognate multiprotein complexes (151, 152). It will therefore be interesting to determine whether similar mechanisms of regulation target other DUBs, safeguarding their proper folding, localization, and stabilization.

**Regulation of DUBs by other post-translational modifications**

**Regulation of DUB activity and function by phosphorylation**

Owing to their involvement in controlling a wide spectrum of cellular processes, DUBs are regulated by other post-translational modifications including phosphorylation, acetylation, limited proteolysis, hydroxylation, and oxidation (11, 153). Selected examples of DUBs indicate the diversity of post-translational modifications that regulate these enzymes (Fig. 5). Phosphorylation is widespread in eukaryotes and regulates a large variety of cellular signaling processes. Several examples demonstrate how phosphorylation regulates DUB stability, activity, or recruitment, further reinforcing the notion that phosphorylation-mediated signaling is intimately associated with deubiquitination. For instance, the stability of USP4, a DUB that regulates transforming growth factor-β signaling, is regulated by phosphorylation (154). Akt-mediated phosphorylation of USP4 prevents its ubiquitination and promotes its accumulation at the plasma membrane, thereby stabilizing transforming growth factor-β receptor and downstream signaling in the regulation of epithelial to mesenchymal transition (154). Another example of tight control of DUB stability by phosphorylation is observed for USP7, which deubiquitinates and stabilizes the E3 Ub ligase MDM2. Phosphorylation of USP7 by casein kinase 2 promotes its stability, thereby maintaining higher
levels of MDM2. This, in turn, promotes p53 degradation, hence maintaining this transcription factor at basal protein levels (155). However, in response to genotoxic stress, dephosphorylation of USP7 by PPM1G results in its destabilization, leading to MDM2 degradation, stabilization of p53, and upregulation of the p53 transcriptional response (155).

Phosphorylation can also directly regulate DUB enzymatic activity. For instance, phosphorylation of OTUD5 catalytic domain induces conformational changes thereby promoting substrate binding and facilitating deubiquitination (156). Another interesting example is provided by OTUD4 whose phosphorylation occurs near the catalytic domain. OTUD4 phosphorylation can also directly regulate DUB enzymatic activity. For instance, phosphorylation of OTUD5 catalytic domain induces conformational changes thereby promoting substrate binding and facilitating deubiquitination (156). Another interesting example is provided by OTUD4 whose phosphorylation occurs near the catalytic domain. OTUD4
phosphorylation, in conjunction with an adjacent Ub-binding domain, promote deubiquitination of K63-linked Ub chains. This mechanism is used by OTUD4 to target MyD88 for deubiquitination and to dampen NF-κB activation in response to Toll-like receptor signaling (157). The DUB OTULIN (described above) can also be subjected to phosphorylation during genotoxic stress, this phosphorylation event seems to be required for the association with and deubiquitination of β-catenin. This, in turn, leads to the noncanonical activation of the Wnt signaling pathway (158).

Phosphorylation is also critical for the BAP1-mediated response to genotoxic stress. BAP1 promotes the recruitment of BRCA1 and RAD51 to sites of DNA double-strand breaks (DSBs) and promotes homologous recombination-mediated DNA repair (9, 32, 159–161). Moreover, BAP1 is directly recruited to genomic regions in the vicinity of DSBs, which promotes the deubiquitination of H2AK119ub on the chromatin (9, 32, 159–161) (Fig. 6A). BAP1 is phosphorylated on six serine/threonine residues following ionizing radiation and mutation of these residues inhibits BAP1 recruitment to DSB sites. However, whether BAP1 phosphorylation involves DUB conformational changes or recruitment of additional proteins remain to be determined.

Altogether, the studies described above emphasize the versatility of phosphorylation in regulating the activity of DUBs and their interaction with other factors. In addition, a survey of the DUB repertoire of phosphorylation sites (PhosphoSite database) shows that DUBs are extensively phosphorylated on multiple domains. This suggests that an intricate crosstalk between multiple signaling pathways tightly orchestrates DUB function.

**Regulation of DUB activity and function by acetylation**

Another prominent example of DUB regulation by post-translational modifications is illustrated by acetylation of OTUD3 in the context of innate immune surveillance (162). Following infection by RNA viruses, the mitochondrial antiviral-signaling protein MAVS undergoes K63-linked polyubiquitination, which is essential for its multimerization and activation of the host antiviral response (163–165). Remarkably, acetylation of OTUD3 on lysine 129 (K129) triggers deubiquitination of K63-polyubiquitination chains on MAVS thereby blocking the antiviral response (162) (Fig. 6B). While OTUD3 acetylation promotes its DUB activity, its deacetylation by SIRT1 inhibits MAVS deubiquitination following viral infection, thus promoting activation of innate immune signaling (162). K129 is found in the variable loop of the OTU catalytic domain, i.e., within an acetylation motif conserved throughout OTUD3 orthologues. Acetylation of this residue abolishes its positive charge, promoting substrate binding. Thus, OTUD3 provides a noteworthy example of direct regulation of DUB catalytic activity by acetylation, with important implications for the antiviral response.

Together, these investigations emphasize the versatility of phosphorylation or acetylation in mediating DUB regulation and suggest that an intricate crosstalk might take place between signaling pathways in order to provide a strict regulation of DUB function.

**Regulation of DUB by atypical post-translational modifications**

In addition to mechanisms involving classical post-translational modifications, other biochemical processes that coordinate DUB activity have been proposed. For instance, UCHL1, a DUB highly expressed in the brain and whose deregulation is associated with neurodegeneration, is subjected to nitrosylation (166). Moreover, its catalytic activity might be affected under conditions of pronounced oxidative stress (Fig. 5). Notably, in vitro studies indicated that oxidation of the three cysteines C90, C152, and C220 inhibits the enzyme and induces significant conformational changes that prevent Ubbinding and, hence, removal from substrates (166). In addition, C152, within the substrate recognition cross-over loop, might act as a scavenger of reactive oxygen species, possibly protecting the catalytic cysteine, although this needs further demonstration in vivo (167). Finally, UCHL1 could also be modified by lipidation (Fig. 5). Under inflammatory conditions, C152 could be subjected to the covalent ligation of cyclopentanone prostaglandins, causing DUB misfolding and neurotoxicity (168, 169).

Consistent with the variety of post-translational modifications regulating DUBs, USP1 is regulated by autocleavage-mediated inactivation during exposure to ultraviolet radiations (170). In particular, USP1 contains an internal Ub-like diglycine (Gly-Gly) motif, which is essential for DUB recognition and catalysis. The autocleavage of USP1 permits the accumulation of monoubiquitinated PCNA and execution of translesion synthesis across UV-induced pyrimidine dimers (170). Of note, USP1 is also regulated by reversible oxidation of cysteine upon oxidative stress, a mechanism proposed to promote PCNA ubiquitination in response to oxidative DNA damage (171). Finally, the DUB Cezanne (OTUD7B) is hydroxylated by the asparaginyl β-hydroxylase factor inhibiting HIF1 (FIH1) within a domain similar to the Ub-associated domain, and this modification blocks Ub-binding, although the significance of this event remains unknown (172).

In summary, the above examples illustrate the relevance of post-translational modifications and their crosstalk in the dynamic regulation of DUB function.

**Concluding remarks**

As outlined in this review, the proper regulation of Ub signaling is of crucial importance for the maintenance of cellular homeostasis. Indeed, humans express more than 600 E3-Ub ligases and about 100 DUBs that regulate thousands of Ub-modified sites (173–175). A substantial body of evidence now indicates that DUBs mediate multiple, highly
orchestrated mechanisms to counterbalance ubiquitination events and regulate Ub signaling. We have summarized diverse control systems that regulate the catalytic activity of DUBs as well as their interaction with E3 ligases and substrates. Deregulation of DUB function is therefore expected to profoundly perturb cellular processes. Indeed, mutation of DUB genes or dysfunction of their protein homeostasis are increasingly associated with major pathologies such as neurodegenerative diseases (USP25, UCHL1, OTUB1, and AT3) (176–181), cancers (BAP1, CYLD, USP46, USP28, and PSMD14) (32, 182–186) and inflammation (A20, CYLD, USP7, and USP47) (187–191). To properly target these enzymes in the clinic, several wide-ranging questions remain to be addressed: (i) What is the full spectrum of substrates modified by each DUB? (ii) What is the complete DUB interactome and how dynamic is it? (iii) To what extent ubiquitination and deubiquitination reactions are spatiotemporally interconnected? and (iv) How the inhibition of DUBs affects physiological processes at the organismal level. Undoubtedly, with the development of new tools and
biotechnologies, there are many exciting prospects for the upcoming decades.

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Abbreviations—The abbreviations used are: CTD, C-terminal domain; DEUBAD, DEUBiquitinate ADaptator; DUB, deubiquitinate; JAMM, JAB1/MPN/MOV34 metalloenzymes; MINDY, motif-interacting with ubiquitin-containing novel DUB family; MJD, Machado–Joseph domain-containing proteases; MAVS, mitochondrial antiviral signaling protein; NLS, nuclear localization signal; OTU, ovarian tumor proteases; TRAF, tumor necrosis factor receptor–associated factor; UCH, ubiquitin carboxy-terminal hydrolases; USP, ubiquitin-specific proteases; Ub, ubiquitin; UBL, ubiquitin-like; ULD, UCHL5-like domain; ZUP1, zinc-finger-containing ubiquitin peptidase 1.

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