JBC REVIEWS

Mechanisms orchestrating the enzymatic activity and cellular functions of deubiquitinases

Received for publication, November 12, 2021, and in revised form, June 13, 2022 Published, Papers in Press, June 25, 2022,
https://doi.org/10.1016/j.jbc.2022.102198

Benjamin Estavoyer[1,‡], **Clémence Messmer**[1,‡], **Mohamed Echbicheb**[1], **Christopher E. Rudd**[2,3], **Eric Milot**[3,4], and **El Bachir Affar**[1,3,*]

From the [1]Laboratory for Cell Signaling and Cancer, and [2]Laboratory for Cell Signaling in Immunotherapy, Maisonneuve-Rosemont Hospital Research Center, Montréal, Québec, Canada; [3]Department of Medicine, University of Montréal, Montréal, Québec, Canada; [4]Laboratory for Malignant Hematopoiesis and Epigenetic Regulation of Gene Expression, Maisonneuve-Rosemont Hospital Research Center, Montréal, Québec, Canada

Edited by George DeMartino

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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[‡] These authors contributed equally to this work.

[*] For correspondence: El Bachir Affar, el.bachir.affar@umontreal.ca.

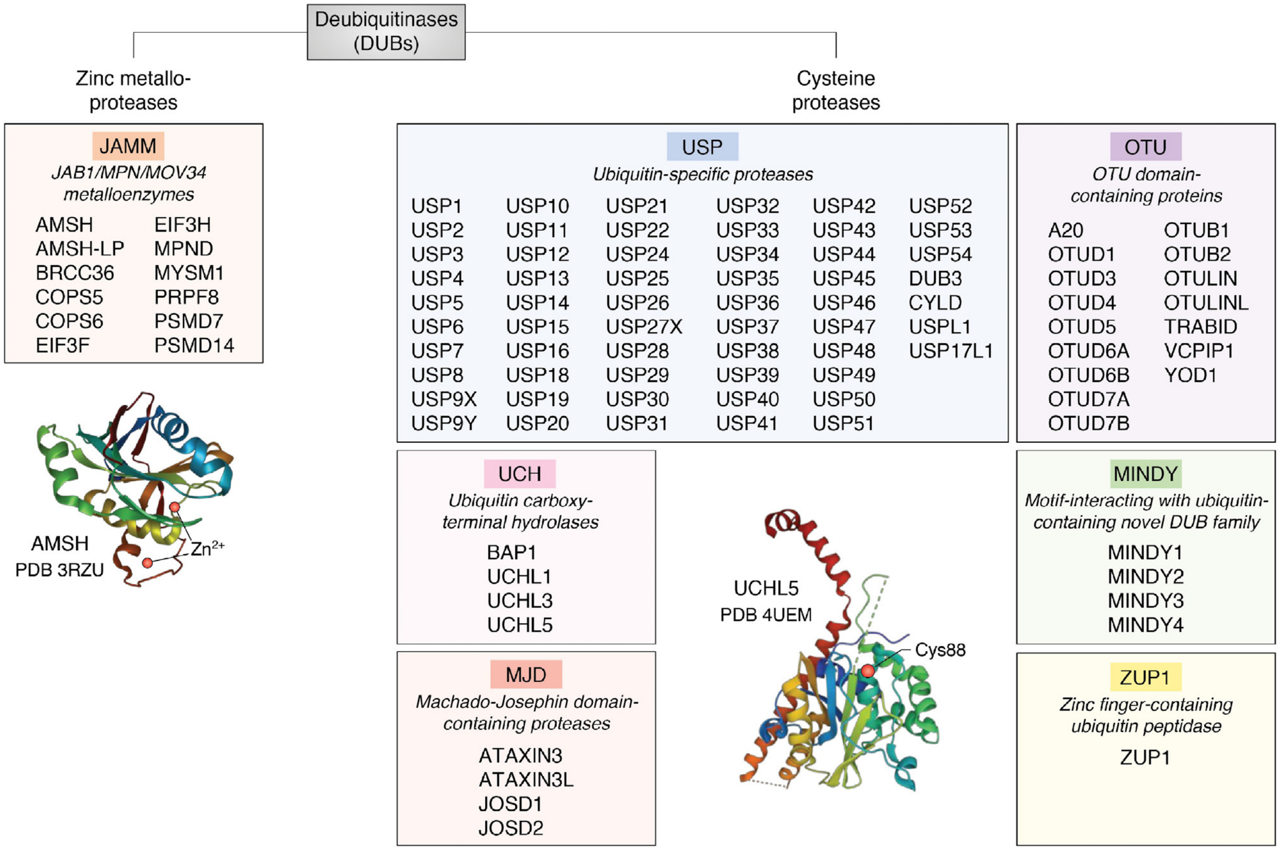


Figure 1. The deubiquitinase (DUB) superfamily. DUBs are classified into seven families: USP, OTU, MJD, UCH, MINDY, JAMM, and ZUP1. The JAMM family are zinc metalloproteases DUBs. The other DUB families are cysteine proteases. JAMM, JAB1/MPN/MOV34 metalloenzymes; MINDY, motif-interacting with ubiquitin-containing novel DUB family; MJD, Machado–Josephin domain-containing proteases; OTU, OTU domain-containing proteins; UCH, ubiquitin carboxy-terminal hydrolases; USP, ubiquitin-specific proteases; ZUP1, zinc finger-containing ubiquitin peptidase 1. AMSH PDB (3RZU), UCHL5 PDB (4UEM).

activity as well as coordinating protein interactions and multiprotein 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. 2*A*). 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. 2*B*). 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 (Fig. 2*B*). This event then favors USP7 stabilization into a DUB competent state (37–39, 41). Interestingly, an additional layer of regulation that involves interacting partners, such as the GMP-synthetase, is likely to be critical for USP7 capacity to target different substrates (42, 43). Indeed, GMP-synthetase

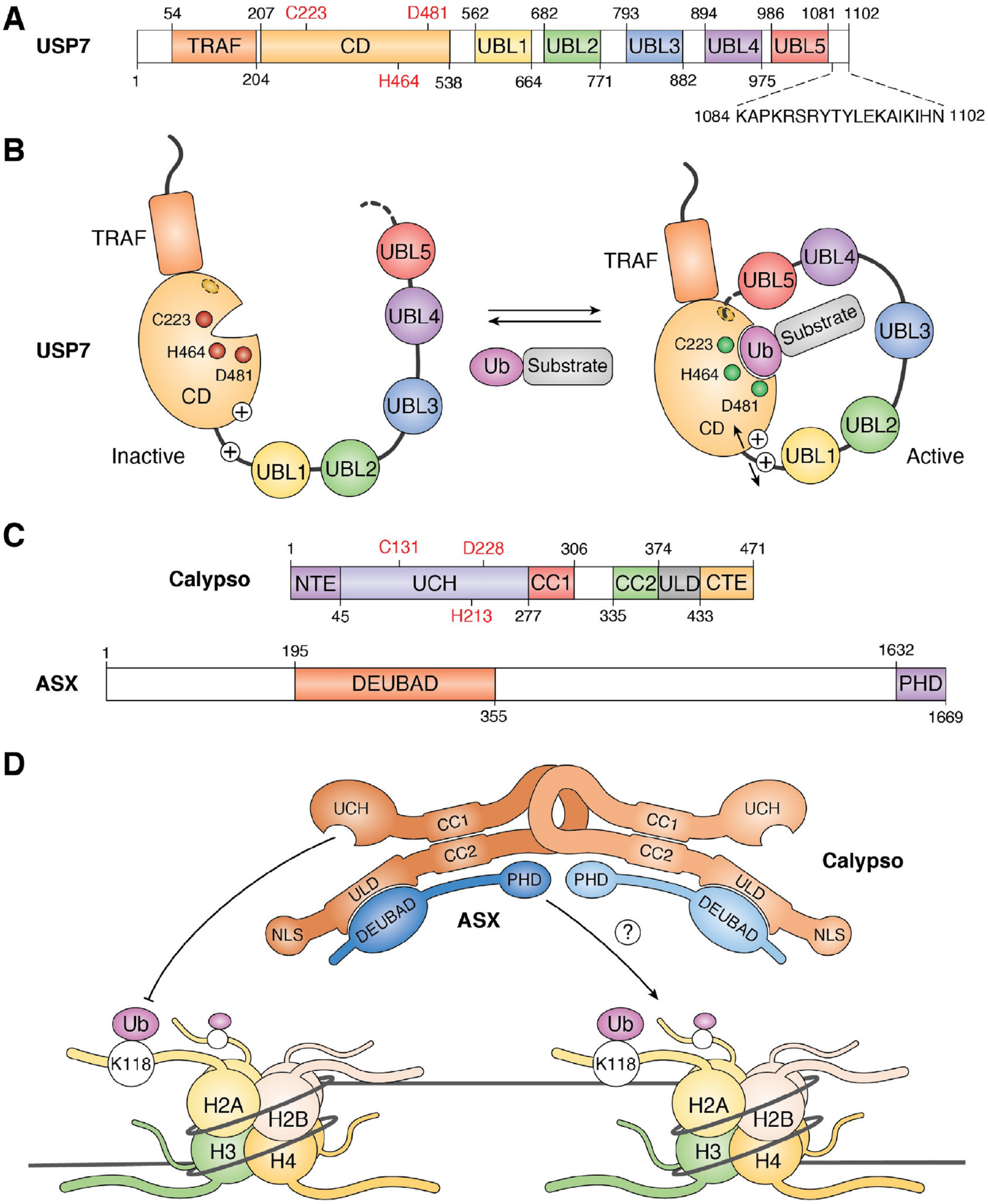


Figure 2. DUB regulation by intramolecular interactions and self-assembly. *A*, schematic representation of USP7 domain organization and boundaries. USP7 catalytic triad is shown in *red* as well as the C-terminal tail (K1084 to N1102) required for its activation in *black*. *B*, USP7 self-activation. 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 stochiometric 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 homeodomain; TRAF, tumor necrosis factor receptor–associated factor; Ub, ubiquitin; UBL, ubiquitin-like; UCH, ubiquitin carboxy-terminal hydrolase; ULD, UCHL5-like domain.

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).

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. 2*C*). 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. 2*D*). 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 DEUBAD (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. 2*D*). 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

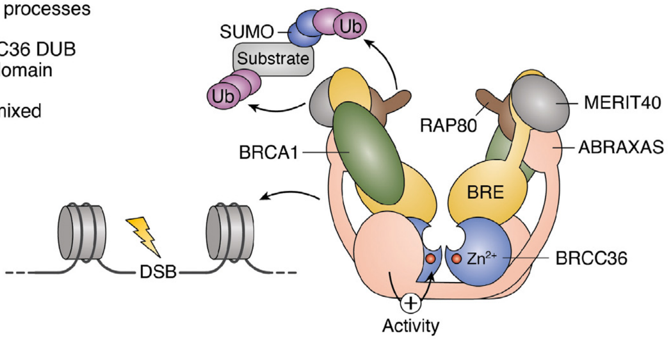
DUB-mediated inhibition of ubiquitination by diverting E2s from E3s

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).

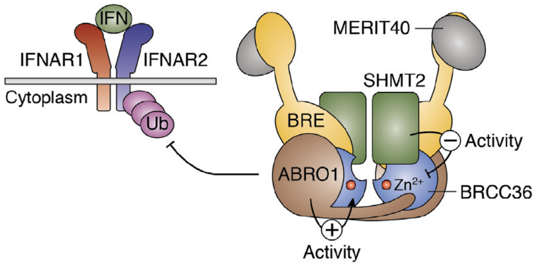
DUBs also assemble into multiprotein complexes wherein they simultaneously interact with both E2 Ub-conjugating enzymes and E3 Ub ligases. For instance, the DUB Ataxin3 (AT3), whose polyglutamine expansion underlies the Machado–Joseph neurodegenerative disorder (63), interacts with UBC7 and UBCH7 E2 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 UBCH7-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

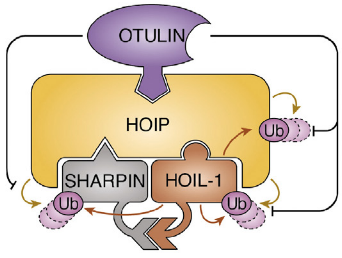
Factors	Subunit organization and function
BRCA1-A complex (JAMM) / *DNA repair*	Refs: 108, 109, 192, 193
BRCC36	K63 poly-Ub chain DUB involved in several biological processes
ABRAXAS	Cofactor involved in BRCA1-A dimerization and BRCC36 DUB activity through its interaction with the JAMM/MPN+ domain
RAP80	Interacts with ABRAXAS, BRE, MERIT40 and binds mixed SUMO/K63 Ub chains
BRE	Interacts with MERIT40, mediates RAP80 integration and BRCA1 sequestration, and binds Ub
MERIT40	Mediates RAP80 integration and binds Ub
BRCA1	Promotes homologous recombination



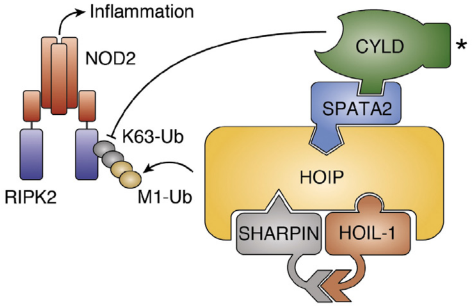
Factors	Subunit organization and function
BRISC complex (JAMM) / *Immunity, mitosis, hematopoiesis*	Refs: 102, 105, 108, 109
BRCC36	K63 poly-Ub chain DUB involved in several biological processes
ABRO1	Cofactor involved in BRCC36 DUB activity through its interaction with JAMM/MPN+ domain and recruits SHMT2
BRE	Involved in SHMT2 recruitment, interacts with ABRO1, MERIT40, and binds Ub
MERIT40	Scaffold subunit which binds Ub
SHMT2	Blocks BRCC36 DUB activity through the exclusion of Ub chains from its catalytic site



Factors	Subunit organization and function
OTULIN-LUBAC complex (OTU) / *Cell death, immunity*	Refs: 74, 80, 81, 83, 88, 194
OTULIN	Met1 poly-Ub chain DUB. Stabilizes the LUBAC complex by promoting its poly-deubiquitination
HOIP	Subunit of the LUBAC complex and a RBR (RING-Between-RING) E3 ligase that catalyzes Met1-Ub-linked ubiquitination. It also interacts with OTULIN
HOIL-1	Subunit of the LUBAC complex and a RBR E3 ligase that monoubiquitinates 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



Factors	Subunit organization and function
CYLD-LUBAC complex (USP) / *Cell death, immunity*	Refs: 74, 90, 91, 194, 195
CYLD	Met1/K63 poly-Ub chain DUB
SPATA2	Required for CYLD recruitment to its target substrates and stimulates its activity. Also acts as a bridging factor between CYLD and HOIP
HOIP	Catalyzes Met1-Ub-linked ubiquitination. Also acts as a scaffold through its interaction with SPATA2
HOIL-1	Subunit of the LUBAC complex and a RBR E3 ligase that monoubiquitinates 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



*Complex homodimerization

Figure 3. DUB complexes: composition, regulation, and complexes.[32, 46, 51–58, 74, 80, 81, 83, 88, 90, 91, 102, 105, 108–110, 112–121, 124–126, 129, 132, 133, 141, 159–161, 182, 192–200] ABRO1, abraxas brother 1; ADRM1, adhesion-regulating molecule 1; AR, androgen receptor; ASXL, additional sex-combs like; BRCA1, breast cancer 1; BAP1, BRCA1-associated protein 1; BRCC36, BRCA1/BRCA2-containing complex subunit 3; BRISC, BRCC36-containing isopeptidase complex; FANCI, Fanconi anemia complementation group I; FANCD2, Fanconi anemia complementation group D2; HAT, histone acetyltransferase; HCF-1, host cell factor 1; HOIP, HOIL-1L interaction protein; IFNAR, interferon alpha and beta receptor; JAMM, JAB1/MPN/MOV34 metalloprotease; LUBAC, linear ubiquitin assembly complex; MERIT40, mediator of RAP80 interactions and targeting subunit of 40 kDa; NFRKB, nuclear factor related to kappaB binding protein; NOD2, nucleotide binding oligomerization domain containing 2; OGT, O-linked N-acetylglucosamine transferase; PHLPP, PH domain leucine-rich repeat protein phosphatase; RAP80, receptor-associated protein 80; RBR, RING between RING; SHMT2, serine hydroxymethyltransferase 2; RIPK2, receptor interacting serine/threonine kinase 2; SAGA, Spt-Ada-Gcn5 acetyltransferase; Sgf11/73, SAGA-associated factor 11/73; Tra1, transcription-associated protein 1; TNKS, tankyrase; UAF1, USP1 associated factor 1; UCH, ubiquitin C-terminal hydrolase; USP, ubiquitin-specific protease; WDR20, WD repeat domain 20.

DUB complexes: Composition, regulation, and functions

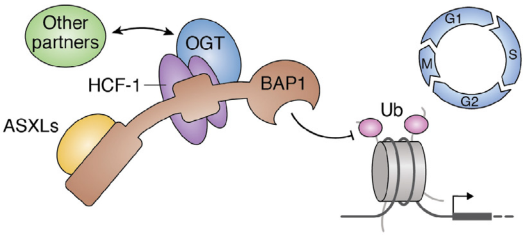
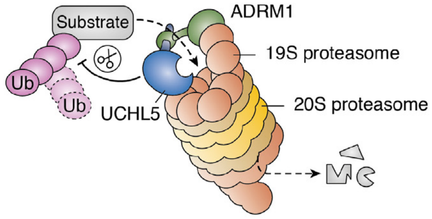
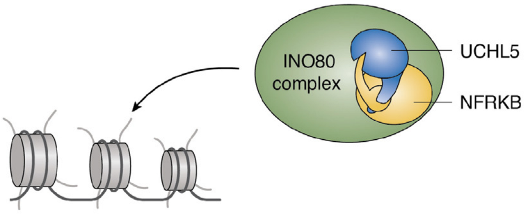
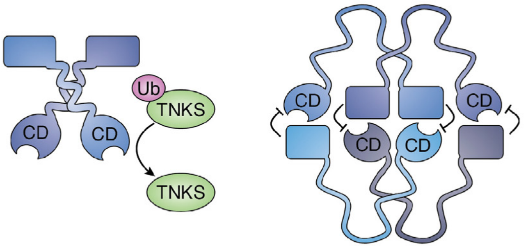
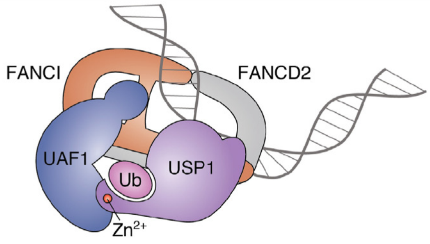
Factors	Subunit organization and function	Complex structure
BAP1 complex (UCH) / *DNA repair, transcription, cell cycle, cell death*		Refs: 32, 46, 132, 133, 141, 159-161
BAP1	DUB of H2AK119. Also involved in the deubiquitination of other substrates	
ASXLs	Cofactors required for BAP1 DUB activity and stability	
OGT	Catalyzes the *O*-GlcNAcylation of target proteins	
HCF-1	Nuclear coactivator/corepressor which mediates the recruitment of other chromatin modifying enzymes	
UCHL5-RPN13-19S proteasome (UCH) / *Proteasomal substrate degradation, cell cycle*		Refs: 51-54, 110, 112, 113
UCHL5	K48 poly-Ub chain DUB	
ADRM1 (RPN13)	19S proteasomal subunit. Increases UCHL5 DUB activity by stabilizing its Ub binding surface and positioning the ULD and the crossover loop (ASCL) in an activity-competent conformation	
19S	Regulatory particle of the proteasome, involved in recognition, deubiquitination, and unfolding of polyubiquitinated proteins	
UCHL5-NFRKB-INO80 complex (UCH) / *Chromatin remodeling complex*		Refs: 52-55
UCHL5	K48 poly-Ub chain DUB	
NFRKB (INO80G)	Component of the INO80 complex. Inhibits UCHL5 DUB activity by blocking its Ub docking site and stabilizing its ULD in an inhibitory conformation	
INO80 complex	ATPase chromatin remodeling complex responsible for nucleosome sliding and involved in several biological processes	
USP25 (USP) / *Inflammation, Wnt signaling pathway*		Refs: 56-58, 196
USP25 (Di/tetrameric assembly)	K48 and K63-linked chain DUB whose regulation is coordinated by oligomerization. USP25 homodimerization leads to its activation whereas its homotetramerization promotes its inhibition	
USP1/UAF1 complex (USP) / *DNA repair, inflammation, AKT signaling pathway*		Refs: 114, 117, 197, 198
USP1	Monoubiquitin and K48/K63-specific DUB involved in deubiquitination of multiple substrates	
UAF1/ WDR48	Activates USP1 through conformational changes in its active site. Also implicated in USP1 stability and recruitment to its substrate	

Figure 3. (Continued).

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

DUB complexes: Composition, regulation, and functions

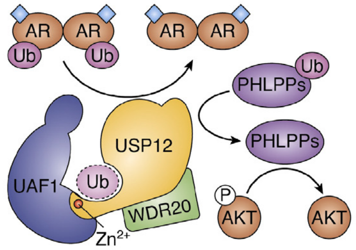
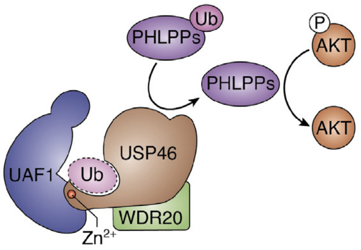
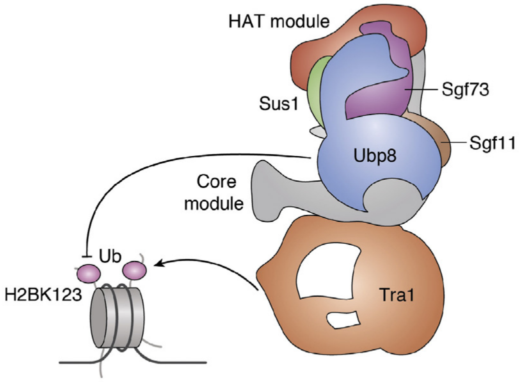
Factors	Subunit organization and function	Complex structure
USP12/UAF1/WDR20 complex (USP) / *Androgen receptor, AKT signaling pathway, immunity, synaptic transmission regulation*		Refs: 115, 116, 119, 121, 198-200
USP12	K48-specific DUB involved in the deubiquitination of several substrates	
UAF1/ WDR48	Activates USP12 through conformational changes (independently of WDR20). Also implicated in substrate recognition	
WDR20	Scaffold subunit which increases USP12 activity via conformational changes (independently of UAF1)	
USP46/UAF1/WDR20 complex (USP) / *AKT signaling pathway, immunity*		Refs: 115, 118, 120, 182, 198
USP46	K48/K63-specific DUB involved in the deubiquitination of several substrates	
UAF1/ WDR48	Activates USP46 through conformational changes (independently of WDR20). Also implicated in substrate recognition	
WDR20	Scaffold subunit which increases USP46 activity via conformational changes (independently of UAF1)	
SAGA complex (USP) - (Yeast) / *Transcription, development*		Refs: 124-126, 129
DUB module	Deubiquitinates H2BK123 (H2BK120 in humans)	
Ubp8	Catalytic subunit of the DUB domain	
Sgf11	Required for Ubp8 DUB activity and recruitment of Sus1 and Ubp8 at the SAGA complex	
Sgf73	Required for Ubp8 DUB activity and its recruitment at the SAGA complex	
Sus1	Required for Ubp8 DUB activity and its recruitment at the SAGA complex	
HAT module	Catalyzes acetylation of histone H3 on multiple residues	
Core module	Scaffold module which stabilizes the SAGA complex, interacts with Tra1, and mediates the assembly of the transcriptional pre-initiation complex (PIC)	
Tra1	Interacts with transcriptional activators and recruits SAGA at gene loci	

Figure 3. (Continued).

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).

Strikingly, while several studies have shown that OTULIN could act on LUBAC-regulated substrates to promote or inhibit downstream signaling (81, 85–87), this DUB seems to act preferentially on components of the LUBAC itself, preventing its autoubiquitination and leading to an increase in its E3-ligase activity (88). It is possible that cell-type and cellular contexts as well as potential Ub-independent functions of OTULIN might account for the apparent discrepancies (89). Thus, further studies are required to fully establish how OTULIN regulates LUBAC-associated signaling events and related cellular processes.

CYLD, which acts specifically toward Met1-Ub and poly-Ub K63 chains, binds indirectly to LUBAC through spermatogenesis-associated protein 2 (SPATA2) (90). SPATA2 is not only required for CYLD recruitment to LUBAC and its substrates (TNFR1 and NOD2) but also for CYLD activation, thus ensuring a spatiotemporal regulation of this DUB (Fig. 3) (90). Furthermore, in order to bind SPATA2, it seems that CYLD needs to be in a dimeric conformation, thereby forming a heterotetrameric complex (90). Surprisingly, a mechanism of interaction similar to that used by OTULIN is also at play. Indeed, a PUB–PIM interaction is engaged with SPATA2, which bridges HOIP with CYLD. Thus, CYLD and OTULIN are mutually exclusive for their interaction with LUBAC and might be finely regulated to exert their functions (90, 91). It will be interesting to define how these DUBs are dynamically interchanged on LUBAC, depending on cellular contexts, and how this coordination regulates cell survival and inflammation.

Finally, it is worth mentioning that DUBs can act cooperatively to stabilize and promote the activity of E3 Ub ligases. For example, the ubiquitin-specific proteases USP7 and USP11 interact with and stabilize several components of the PRC1 complex, the E3 Ub ligase responsible for H2AK119 monoubiquitination, and transcriptional repression of polycomb group target genes, including the INK4a tumor suppressor (92). Remarkably, depletion of USP7 also influences the association of USP11 with chromatin, suggesting an intimate relationship between these two DUBs in PRC1 regulation (92).

In summary, DUBs can dynamically interact with E3 Ub ligases, and this can affect their own enzymatic activity as well as the protein stability or activity of E3 Ub ligases. This not only adds a layer of selectivity for the ubiquitination of substrates but also allows the fine-tuning of signaling events.

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 combs–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. 4*A*) (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 multimonoubiquitinated 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 monoubiquitination. 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

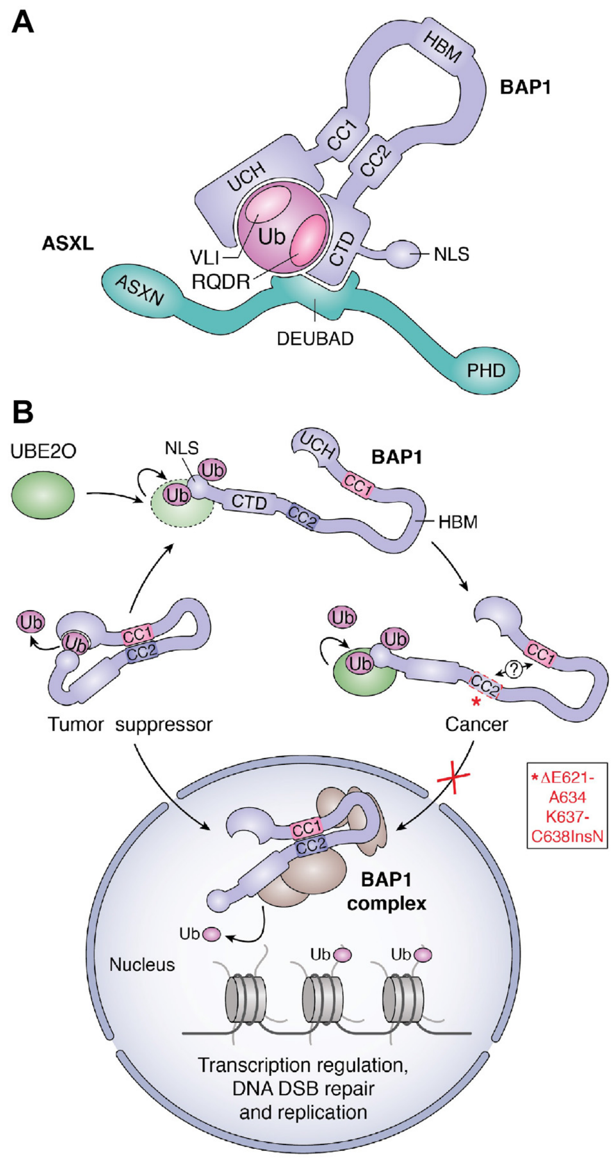


Figure 4. Regulation of BAP1 by intramolecular interactions and self-deubiquitination. *A*, BAP1 intramolecular interactions are required for its ASXLs-mediated regulation. Interactions between the UCH-CC1 and the CTD domains of BAP1 as well as between the CTD and the DEUBAD of ASXLs are essential for the formation of the CUBI (composite ubiquitin-binding interface). This composite interface enables binding of the VLI hydrophobic patch and the RQDR charged patch of ubiquitin to BAP1 UCH domain and CTD, respectively. This assembly results in the activation of BAP1 DUB activity. *B*, regulation of BAP1 by self-deubiquitination. UBE2O is a E2 Ub-conjugating-E3 Ub ligase hybrid enzyme that multimonoubiquitinates BAP1 in its NLS region. The CC2 region of BAP1 interacts with the UCH-CC1 domains. This event brings the NLS close enough to the UCH catalytic domain facilitating NLS deubiquitination. BAP1 is then able to translocate into the nucleus and act as a tumor suppressor. Cancer mutations localized in the CC2 domain promote BAP1 association with UBE2O and lead to the disruption of BAP1 self-deubiquitination. As a consequence, BAP1 is sequestered in the cytoplasm. BAP1, BRCA1-associated protein 1; CC1/2, coiled-coil 1/2; CTD, C-terminal domain; DEUBAD, DEUBiquitinase ADaptator; HBM, HCF-1 binding motif; NLS, nuclear localization signal; PHD, plant homeo-domain; Ub, ubiquitin; UCH, ubiquitin carboxy-terminal hydrolase.

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.

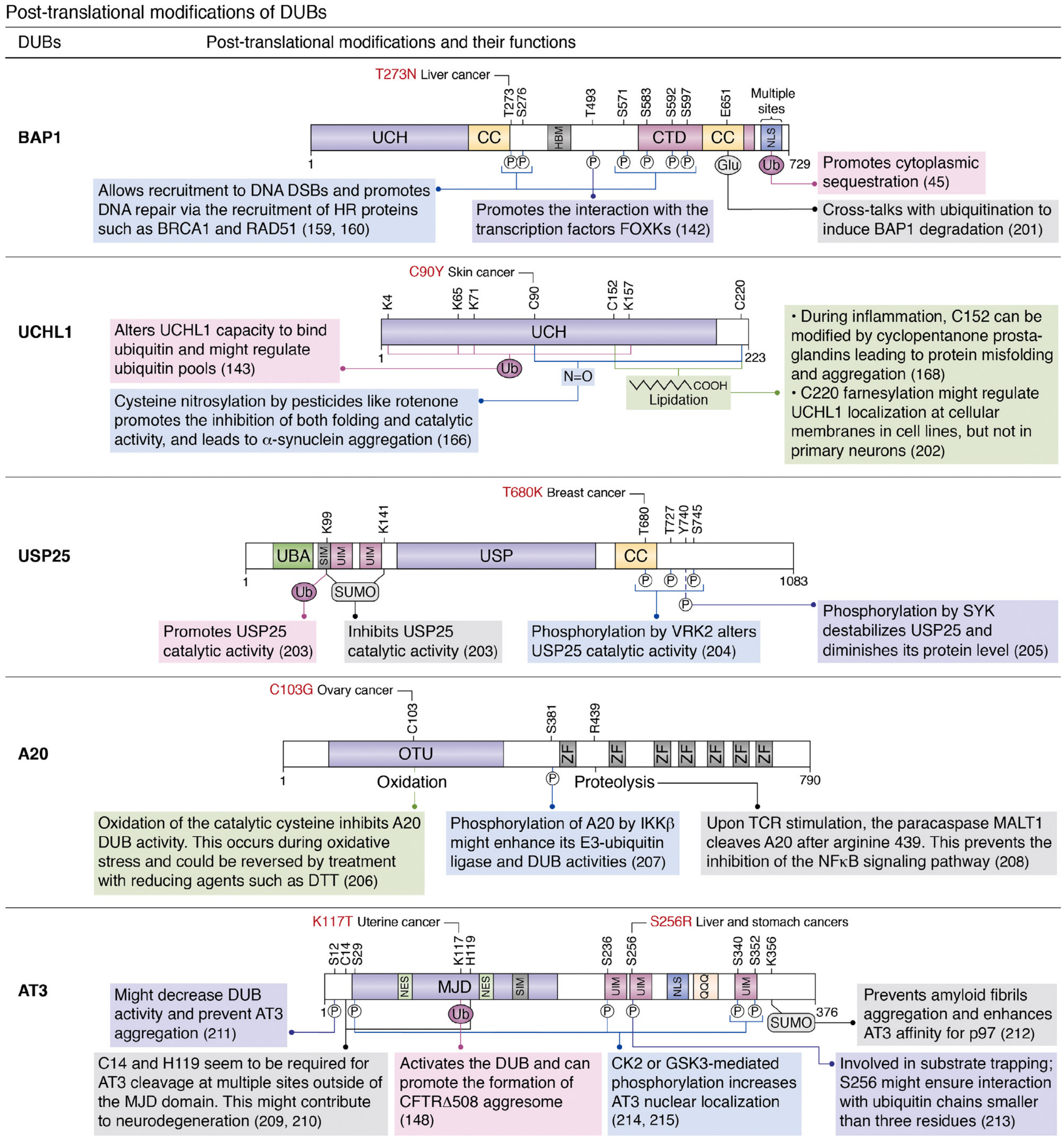
Regulation of DUB localization by ubiquitination

Self-deubiquitination of BAP1 provides a notable example of DUB regulation at the level of subcellular localization (Fig. 4*B*). 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. 4*B*). 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



BAP1:

Allows recruitment to DNA DSBs and promotes DNA repair via the recruitment of HR proteins such as BRCA1 and RAD51 (159, 160)

Promotes the interaction with the transcription factors FOXKs (142)

Promotes cytoplasmic sequestration (45)

Cross-talks with ubiquitination to induce BAP1 degradation (201)

UCHL1:

Alters UCHL1 capacity to bind ubiquitin and might regulate ubiquitin pools (143)

Cysteine nitrosylation by pesticides like rotenone promotes the inhibition of both folding and catalytic activity, and leads to α-synuclein aggregation (166)

- During inflammation, C152 can be modified by cyclopentanone prostaglandins leading to protein misfolding and aggregation (168)
- C220 farnesylation might regulate UCHL1 localization at cellular membranes in cell lines, but not in primary neurons (202)

USP25:

Promotes USP25 catalytic activity (203)

Inhibits USP25 catalytic activity (203)

Phosphorylation by VRK2 alters USP25 catalytic activity (204)

Phosphorylation by SYK destabilizes USP25 and diminishes its protein level (205)

A20:

Oxidation of the catalytic cysteine inhibits A20 DUB activity. This occurs during oxidative stress and could be reversed by treatment with reducing agents such as DTT (206)

Phosphorylation of A20 by IKKβ might enhance its E3-ubiquitin ligase and DUB activities (207)

Upon TCR stimulation, the paracaspase MALT1 cleaves A20 after arginine 439. This prevents the inhibition of the NFκB signaling pathway (208)

AT3:

Might decrease DUB activity and prevent AT3 aggregation (211)

Prevents amyloid fibrils aggregation and enhances AT3 affinity for p97 (212)

C14 and H119 seem to be required for AT3 cleavage at multiple sites outside of the MJD domain. This might contribute to neurodegeneration (209, 210)

Activates the DUB and can promote the formation of CFTRΔ508 aggresome (148)

CK2 or GSK3-mediated phosphorylation increases AT3 nuclear localization (214, 215)

Involved in substrate trapping; S256 might ensure interaction with ubiquitin chains smaller than three residues (213)

Figure 5. Post-translational modifications of DUBs.[45, 142, 143, 148, 159, 160, 166, 168, 201–215] AT3, ataxin3; BAP1, BRCA1-associated protein 1; CC, coiled-coil; CFTR, cystic fibrosis transmembrane conductance regulator; CK2, casein kinase 2, CTD, C-terminal domain; DTT, dithiothreitol; DUB, deubiquitinase; FOXK, forkhead box class K; Glu, glutamylation; GSK3, glycogen synthase kinase 3; HBM, HCF-1 binding motif; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; MJD, Machado–Joseph deubiquitinases; NFκB, nuclear factor kappa B; NES, nuclear exporting signal; NLS, nuclear localization signal; P, phosphorylation; QQQ, poly-Q region; SUMO, SUMOylation; SIM, SUMO-interacting motif; Ub, ubiquitin; UBA, Ub-associated domain; UCH, Ub-C terminal hydrolase; UIM, Ub-interacting motif; USP, ubiquitin carboxyl-terminal hydrolase; ZF, zing finger.

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, 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 events. Phosphorylation of OTULIN tyrosine 56 (Y56P) in the PIM domain prevents its binding with HOIP-LUBAC, therefore impairing OTULIN-mediated LUBAC regulation (83). 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. 6*A*). 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 immunity 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. 6*B*). 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 Ub-recognition 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

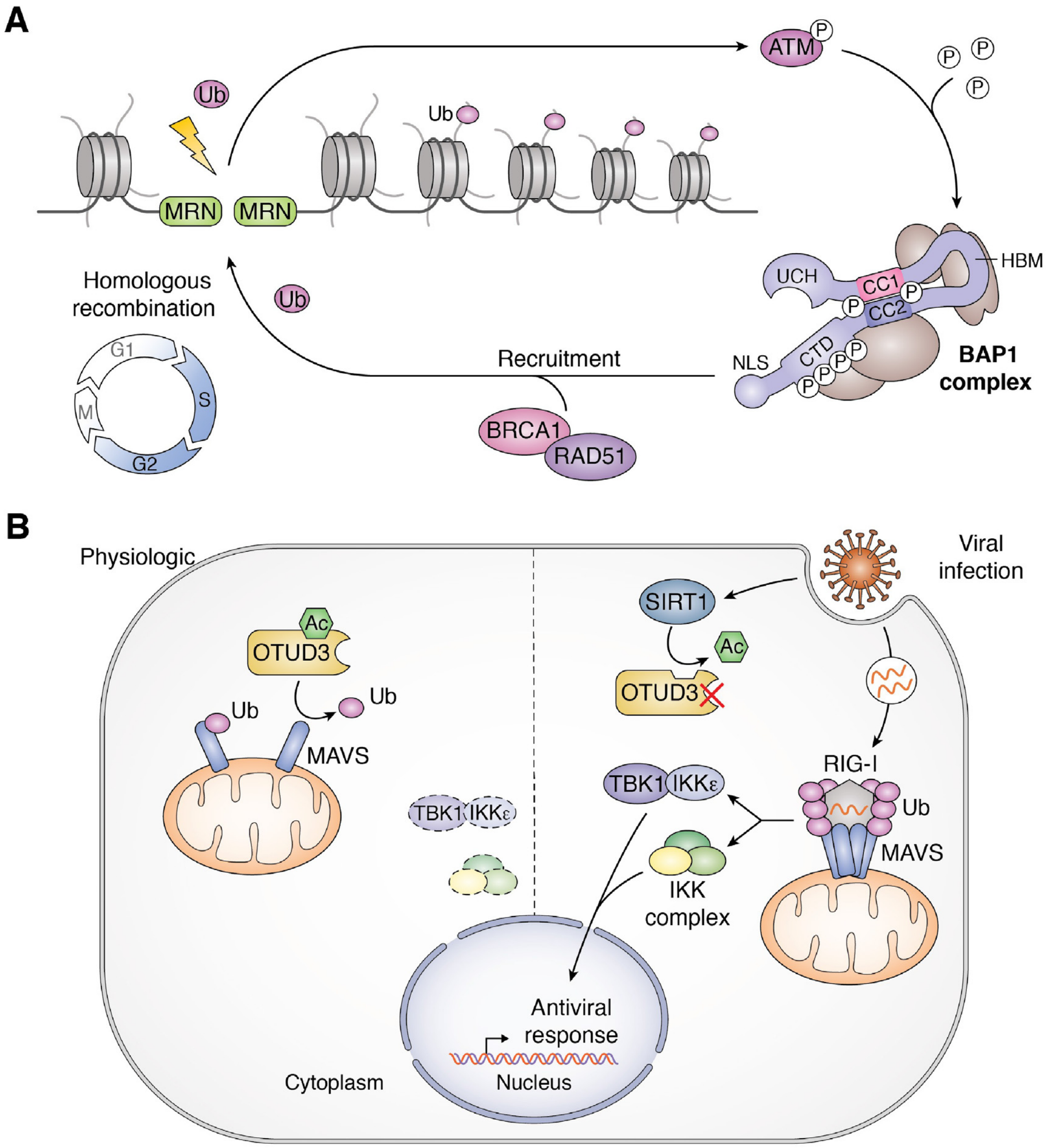


Figure 6. DUB regulation by post-translational modifications. *A*, phosphorylation of BAP1 during the DNA damage response. Upon ionizing-irradiation, DNA double-stand breaks (DSBs) are recognized by the MRN complex resulting in ATM activation. ATM then phosphorylates BAP1 leading to its recruitment at the DSB sites. In turn, BAP1 recruits homologous recombination (HR) proteins such as BRCA1 and RAD51 to promote HR. *B*, acetylation of OTUD3 in innate immunity. Under normal conditions, OTUD3 is acetylated on its lysine 129 (K129-Ac). K129-Ac enhances OTUD3 DUB activity toward the poly-K63 ubiquitin chains of the immune protein MAVS. This mechanism prevents the aggregation of MAVS and activation of the innate immune response. However, upon viral infection, the deacetylase SIRT1 is recruited to OTUD3 to catalyze its deacetylation and inactivation. As a consequence, MAVS becomes poly-ubiquitinated and interacts with RIG-I, leading to its aggregation. This ultimately leads to the activation of downstream immune signaling pathways involved in the antiviral response. ATM, ataxia telangiectasia mutated; BAP1, BRCA1-associated protein 1; IKK, IκB kinase; MAVS, mitochondrial anti-viral signaling protein; MRN, MRE11/RAD50/NBS1; OTUD3, OTU-domain containing protein 3; RIG-I, retinoic acid-inducible gene I; SIRT1, sirtuin 1; TBK1, TANK-binding kinase 1; Ub, ubiquitin.

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 spatio-temporally 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.

Acknowledgments—We thank Dr Nathalie Labrecque and Dr Elliot Drobetsky for comments and suggestions on the manuscript.

Author contributions—B. E., C. M., and E. B. A conceptualization; B. E. and C. M. data curation; E. B. A. writing-original draft; B. E., C. M., M. E., C. E. R., E. M., and E. B. A. writing-review and editing.

Funding and additional information—This work was supported by the Canadian Institutes of Health Research.

Conflicts of interest—The authors declare that they have no conflicts of interest with the content of this article.

Abbreviations—The abbreviations used are: CTD, C-terminal domain; DEUBAD, DEUBiquitinase ADaptator; DUB, deubiquitinase; JAMM, JAB1/MPN/MOV34 metalloenzymes; MINDY, motif-interacting with ubiquitin-containing novel DUB family; MJD, Machado–Josephin 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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