Published in final edited form as:

Curr Opin Cell Biol. 2008 April ; 20(2): 156–163.

Reverse the curse - the role of deubiquitination in cell cycle control

Ling Song¹ and Michael Rape^{1,#}

¹*Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, USA*

Abstract

Reversible protein ubiquitination is a crucial mechanism regulating the progression through the eukaryotic cell cycle. Ubiquitin-dependent signaling is terminated by specific deubiquitinating enzymes (DUBs), which now are known to be integral components of the core cell cycle machinery and cell cycle checkpoints. The importance of DUBs for cell cycle control is underscored by their frequent misregulation in cancer. Here, we discuss the role of deubiquitinating enzymes in controlling proliferation.

Introduction

The complexity of multicellular organisms depends on carefully orchestrated developmental programs that ensure that cells divide and differentiate at the right time and place. In eukaryotes, cell division is driven by oscillations in the activities of cyclin-dependent kinases (CDKs), which are coupled to the development of the organism by growth- and differentiation factor signaling. This core cell cycle machinery is controlled by checkpoint signaling networks, which continuously scrutinize cells for aberrations and initiate necessary repair responses. The dynamic interplay between core machinery and checkpoints protects multicellular organisms from unscheduled proliferation and cancer.

During the last two decades, ubiquitination has been identified as a crucial regulator of both the core cell cycle machinery and cell cycle checkpoints. Ubiquitinated proteins are often targeted for degradation by the 26S proteasome, the irreversible nature of which is at the heart of the unidirectional progression through the cell cycle program [1,2]. Reversible ubiquitination, however, can also trigger endocytosis of growth factor receptors or modulate the activity of kinases and transcription factors, thereby empowering cells to interpret signals from their environment or checkpoints. It is not surprising that the misregulation of any of these processes can have dire consequences for cell division in multicellular organisms.

Ubiquitination is a covalent modification, which involves the formation of an isopeptide bond between the carboxy-terminus of ubiquitin and the ϵ -amino group of a lysine residue within the acceptor protein [1]. This reaction is carried out by an enzymatic cascade involving at least three different activities (E1, ubiquitin activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin-protein ligase). Substrates can be decorated with a single ubiquitin moiety (monoubiquitination), or with ubiquitin chains (multiubiquitination). Depending on the lysine residue of ubiquitin, which is used for chain formation, chains differ in structure and function. K48-linked ubiquitin chains target proteins for degradation by the 26S proteasome, whereas

to whom correspondence should be addressed: mraper@berkeley.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

K63-linked ubiquitin chains fulfill mostly non-proteolytic roles in DNA repair or other signaling processes.

Similar to other covalent modifications, such as phosphorylation or methylation, ubiquitination is reversible. The human genome harbors approximately 100 deubiquitinating enzymes (DUBs), which cleave ubiquitin off substrates and thereby terminate ubiquitin-dependent signaling [3]. Reminiscent of the role of phosphatases in signal transduction, DUBs have only lately been recognized as essential and specific components of the ubiquitin pathway. A flurry of recent papers has pointed to pivotal DUB functions in cell cycle control and has tightly linked the misregulation of DUBs to tumorigenesis. Here, we will review the function of DUBs in cell cycle control, and when appropriate discuss their relevance for cancer and drug development.

Mechanism and regulation of deubiquitinating enzymes

The majority of human DUBs are cysteine-proteases that based on homologies between their catalytic domains can be clustered into four subfamilies: ubiquitin-specific proteases (Usp), ubiquitin carboxy-terminal hydrolases (UCH), ovarian tumor-like proteases (OTU), and Machado-Jakob-Disease proteases (MJD). They all contain an active-site cysteine residue, which is part of a catalytic triad comprised of cysteine, histidine, and aspartate [4-7]. Although DUBs of the different subfamilies have surprisingly distinct three-dimensional structures, they share a conserved spatial arrangement of the residues of the catalytic triad. In addition to cysteine proteases, one class of DUB-metalloenzymes has been described [8,9,18]. These enzymes contain a JAMM-domain coordinating a catalytically important Zn^{2+} -ion. The human genome harbors 58 Usps, 4 UCHs, 14 OUT-, 5 MJD-, and 14 JAMM domain-DUBs [3]. Interestingly, deubiquitination can also be carried out in human cells by viral proteins following a viral infection [10,11]. These viral DUBs have been identified mainly by innovative functional proteomic approaches [12], and do not share much sequence or structural similarity with human DUBs [13].

Most DUBs use a negatively charged cleft on the surface of their catalytic domain to recognize the extended carboxy-terminus of ubiquitin [4,5]. In addition, they often interact with large surfaces close to the hydrophobic patch of ubiquitin. The binding of ubiquitin can trigger major structural rearrangements of the catalytic DUB-domain, resulting in the optimal orientation of catalytic residues towards the scissile isopeptide bond [14]. Many DUBs contain additional ubiquitin-binding motifs, such as UBA, ZnF-UBP, or UIM domains, which potentially endow DUBs with specificity towards ubiquitin chains of defined length or linkage [3,15]. DUBs can also recognize motifs within the ubiquitinated proteins using additional domains, which in some cases are connected with the catalytic domain by flexible linkers [16,17]. The simultaneous recognition of motifs within substrates and ubiquitin likely increases the affinity and specificity of DUBs towards their substrates.

As with most regulators of cell cycle progression, the activity of DUBs is tightly controlled (for an example see Figure 1). Several DUBs are synthesized and degraded in a cell-cycle dependent manner. Their expression levels peak at cell cycle stages controlled by their activity, such as the S phase DUB Usp1, the G2/M DUB Cyld, or the mitotic DUB Usp44 [19,20,76]. Even if expressed, DUBs often require the incorporation into larger protein complexes to be fully functional. This is certainly the case with yeast Ubp6, human Usp14, and UCH37, which are activated upon interaction with non-catalytic subunits of the 26S proteasome [21-24]. Recent findings with recombinant Usp44, the activation of which requires incubation with cell extracts [20], and with Usp1, which is activated upon binding to the WD40-repeat containing protein UAF1 [25], suggest that this mode of regulation occurs more frequently than originally thought. Besides mediating activation, binding partners also help localize DUBs to specific

sites, such as early endosomes or the cytoskeleton, or channel them into specific pathways [84]. Some DUBs, such as Usp8, Usp44, or Cyld are likely activated by cell cycle-dependent phosphorylation [19,20,26]. By contrast, inactivation of DUBs can occur by proteasomal degradation, dissociation of binding partners, or inhibitory cleavage of the catalytic domain as originally first shown for Usp1 [27]. The inhibitory cleavage of Usp1 requires its own catalytic activity and amino acids within its catalytic domain, which are homologous to the carboxy-terminus of ubiquitin. DUB-activity and specificity, therefore, are controlled by an astonishing variety of mechanisms, attesting to the importance of proper DUB activity for cellular regulation.

Cell-cycle regulation by DUBs

Several studies have now demonstrated the importance of deubiquitinating enzymes for proliferation. Similar to ubiquitin ligases, DUBs participate in cell cycle control at almost every level. They have been proposed to function in such different processes as ubiquitin homeostasis, transcriptional control, or checkpoint regulation. Since we are only beginning to decipher mechanisms of DUB-dependent cell cycle control, novel functions of these pivotal enzymes are likely to be discovered in the near future. Our current knowledge of selected DUBs, however, already allows us to distill general features of DUB-dependent cell cycle control, as described in the following paragraphs.

Housekeeping DUBs

Ubiquitin is synthesized as a linear fusion to other ubiquitin moieties or to ribosomal proteins [28 and references therein]. Its activation depends on the disassembly of these polyproteins by DUBs, such as isopeptidase T. Strikingly, even though ubiquitin targets other proteins for proteasomal or lysosomal degradation, it itself is relatively stable with a half life of several hours [29,30]. This is also due to the activity of DUBs, which cleave ubiquitin off substrates prior to their translocation into the proteasome or further targeting to the lysosome. A failure of these DUBs to recycle ubiquitin leads to depletion of intracellular ubiquitin and delays cell cycle progression [30,31]. The effects of DUB inhibition are exacerbated under stress conditions, when increased ubiquitination and degradation serve to remove misfolded and potentially deleterious proteins from the cellular pool [28]. A failure of DUBs to cleave ubiquitin off proteins that are already engaged with the 26S proteasome impairs proteasome function and, consequently, cell cycle progression [8]. Such DUBs that indirectly control proliferation by maintaining ubiquitin levels or proteasome activity may be referred to as housekeeping DUBs. As cancer cells continuously experience cellular stress, these enzymes represent potential targets for therapeutic intervention.

Regulation of ubiquitin ligases with cell cycle function

DUBs control cell cycle progression more directly by regulating the stability or activity of ubiquitin ligases. Although it might sound surprising, DUBs are often found in stable complexes with ubiquitin ligases, such as Mdm2 or Brca1, the misregulation of which are known to cause aberrant proliferation and cancer. Among several known examples, the co-regulation of the DUB Usp7/Hausp and its binding partner, the ubiquitin ligase Mdm2, is best understood.

Usp7/Hausp was originally isolated as the cellular binding partner of the herpesvirus ICP0 protein, but soon thereafter, it was shown to associate with the ubiquitin ligase Mdm2 and its substrate p53 [32-35]. Usp7/Hausp interacts with both Mdm2 and p53 through its amino-terminal TRAF-like domain [16]. In the absence of substrates, some RING-finger ubiquitin ligases, including Mdm2, modify themselves and consequently trigger their own proteasomal degradation. This constitutes a negative feedback loop, which effectively ties the levels of

ubiquitin ligases to their respective substrates [36]. DUBs are known to be important components of these regulatory networks. In the case of Mdm2, depletion of Usp7/Hausp in cells leads to increased autoubiquitination and premature degradation of Mdm2 even in the presence of p53 [34,35]. The resulting drop in Mdm2 levels strongly impairs the ubiquitination of the Mdm2-target p53, which in turn can accumulate and induce a cell cycle arrest in G1 or G2. Thus, Usp7/Hausp exerts cell cycle control by shielding a critical ubiquitin ligase from unwanted degradation.

Such intimate relations between ubiquitin ligases and deubiquitinating enzymes are surprisingly widespread. The heterodimeric E3 Brca1/Bard1, for example, is found in complexes with two different DUBs, the UCH Bap1 and the JAMM-domain DUB Brcc36 [37,38]. Brcc36 stimulates the catalytic activity of Brca1/Bard1 and cooperates with Brca1/Bard1 in mediating a G2/M-checkpoint arrest. Brca1/Bard1 can be activated by the non-proteolytic ubiquitination of the ubiquitin ligase itself [39]. It is tempting to speculate that Brcc36 increases Brca1/Bard1-activity by protecting it from non-productive or even inhibitory autoubiquitination at sites required for substrate- or E2-binding. A similar non-proteolytic regulation of E3 activity by DUBs is observed during mitosis, when inhibition of the E3 APC/C is maintained by Usp44 [20], as described in more detail below.

The most intimate relationship between DUBs and ubiquitin ligases is illustrated by the A20 protein, which affects proliferation and cell survival by regulating the NF- κ B pathway [40]. In this case, the same polypeptide chain harbors domains with DUB- (OTU-domain) and E3-activity (Zn-fingers). In vivo, A20 preferentially disassembles K63-linked ubiquitin chains, which are crucial for NF- κ B-activation [41]. Following the removal of K63-linked ubiquitin chains from substrates, such as RIP or Nemo, A20 then assembles K48-linked chains on the same substrates and completes their inactivation by targeting them for degradation. The NF- κ B pathway and ubiquitination of RIP is under control of at least one other DUB, Cyld, which is mutated in familial cylindromatosis [42-44]. Combining DUBs and ubiquitin ligases within complexes or even within same polypeptide chain creates effective molecular machines permitting dynamic cell cycle regulation.

Regulation of cell cycle-specific transcription and chromatin structure

Many key cell cycle regulators are synthesized at specific cell cycle stages. Their expression is controlled by transcription factors, such as E2F or c-Myc, the activities of which are tightly coupled to cell cycle progression. Transcription factors are often very short-lived proteins and rapidly degraded by the ubiquitin/proteasome system. In fact, an inverse correlation between transcription factor activity and stability has been described [45]. The transcription factor c-Myc, for example, promotes entry of cells into S phase and is turned over with a half-life of 30 minutes following its ubiquitination by the SCF^{Fbw7} or SCF^{Skp2} ubiquitin ligases [46,47]. The bulk of c-Myc is degraded after ubiquitination by a nucleolar Fbw7-isoform, Fbw7 γ . A prerequisite of c-Myc-degradation is phosphorylation on two sites, T58 and S62. Mutation of these residues or deletion of the Fbw7 tumor suppressor stabilizes c-Myc and results in cancer [48].

The DUB Usp28 was isolated as a factor required for c-Myc-induced apoptosis, but subsequently shown to be equally important for c-Myc's proliferative functions [49]. Usp28 is found in ternary complexes with c-Myc and the Fbw7 α -isoform, in which it protects c-Myc from SCF^{Fbw7 α} -dependent ubiquitination and degradation. Usp28 also interferes with the premature ubiquitination of other Fbw7-substrates, including cyclin E. As described below, Usp28 is an important component of DNA damage checkpoints [50]. Interestingly, DNA damage induces the dissociation of Usp28 from c-Myc and triggers c-Myc-degradation, thereby stalling cell cycle progression after damage [51]. Consistent with a function of Usp28 in promoting proliferation, it is overexpressed in colon and breast carcinoma [49]. Moreover,

Burkitt's lymphoma cells exhibit higher levels of deubiquitination activity, which may contribute to increased steady state levels of c-Myc [52]. Incorporating the substrates into ternary complexes with ubiquitin ligases and DUBs in all likelihood facilitates the dynamic regulation of substrate levels in response to stimuli, such as DNA damage. Inhibiting the DUBs within those complexes, however, could be used to tip the balance towards c-Myc-ubiquitination and degradation, thereby interfering with the proliferation of c-Myc-dependent tumors.

Ubiquitination does not only control the stability, but also the activity of transcription factors [53]. The forkhead transcription factor Foxo4, for example, is activated in response to cellular stress by monoubiquitination [54]. Monoubiquitination of Foxo4 triggers its nuclear translocation and potentially increases the affinity of Foxo4 for transcriptional co-activators or polymerases, which often have ubiquitin-binding domains [54,55]. As a consequence of its activation, Foxo4 stimulates the transcription of the CDK-inhibitor p27, which results in cell cycle arrest. When the cellular conditions have improved and the cell cycle machinery needs to resume, Foxo4 is deubiquitinated by the already described Usp7/Hausp. The deubiquitination of Foxo4 by Usp7/Hausp thus ensures the transient nature of a stress-induced cell cycle arrest [54].

Cell-cycle specific transcription is also indirectly regulated by DUBs that modulate chromatin structure. The accessibility of chromatin for the transcription apparatus is determined by a highly orchestrated interplay of distinct histone modifications, referred to as the histone code. One of these modifications is the transient ubiquitination of histones, which in fact provided the first example of ubiquitination in cells [56,57]. The ubiquitination of histone H2A by the Bmi1/RING1A heterodimeric ubiquitin ligase is an important regulatory event in *HOX* gene expression and X-chromosome inactivation [58]. H2A is deubiquitinated upon progression through mitosis [59], which potentially facilitates histone phosphorylation and chromosome segregation. The mitotic deubiquitination of H2A within intact nucleosomes is catalyzed by Usp16/Ubp-M [60]. Loss of Usp16/Ubp-M increases the amount of ubiquitinated H2A and, consequently, impairs progression of cells through mitosis. Additional DUBs, including the S phase-specific Usp3 [61], limit histone ubiquitination at other stages of the cell cycle. Regulation of transcription and chromatin structure are most intimately connected with each other in the SAGA transcriptional co-activator complex [62]. A subunit of this complex, Ubp8, deubiquitinates histone H2B, which is required for transcriptional control at several loci. The regulation of chromatin structure and transcription is one of the key mechanisms by which DUBs exert cell cycle control.

Regulation of growth factor signaling

The progression through the different stages of the cell cycle program is coordinated with the development of the organism by growth- and differentiation-factor signaling. Both the strength and duration of growth-factor signaling determine whether cells pause in quiescence, progress through the cell cycle, or enter a differentiation program [63]. The signaling is terminated by the endocytosis and lysosomal targeting of ligand-bound growth-factor receptors, which is triggered by their ubiquitination at the plasma membrane [64,65]. Following the sorting of ubiquitinated receptors to early endosomes, further trafficking to the lysosome depends on consecutive ubiquitin-dependent interactions with ESCRT-complexes. Several DUBs play important roles in regulating the endocytic downregulation of activated growth-factor receptors. Among these, Usp8/UbpY interacts with and is phosphorylated by activated epidermal growth factor receptor (EGFR), but its precise function in EGFR-signaling remains to be established. Some siRNA-studies suggest that Usp8/UbpY-dependent deubiquitination of EGFR promotes further trafficking to the lysosome and EGFR degradation [66,67]. By contrast, another siRNA-study and a *USP8* knockout in mice came to the opposite conclusion

that deubiquitination in fact rescues EGFR from degradation and allows its recycling to the plasma membrane [68,69]. This discrepancy may originate from differences in cell types. Alternatively, it might be a consequence of functions of Usp8/UbpY at additional steps of endocytosis, when Usp8/UbpY is known to protect the critical endocytic regulators Hrs and STAM from proteasomal degradation. Consistent with a function in EGFR- and cell cycle regulation, Usp8/UbpY is expressed in a cell cycle-dependent manner [70]. It is absent from quiescent cells, that did not experience growth factor signaling for prolonged periods of time. A potential function of Usp8/UbpY in cell cycle control is supported by an oncogenic fusion between Usp8/UbpY and the PI-3K subunit p85 β , which was isolated from a leukemia patient [71].

Several other DUBs, including yeast Doa4, *Drosophila* Faf, and human AMSH, regulate endocytosis and protein trafficking to lysosomes [72-74]. The *Drosophila melanogaster* protein fat facets (Faf) was in fact one of the first DUBs for which a specific cellular function had been described. Faf binds to the epsin homolog Liquid facets (Lqf). Together, Faf and Lqf control the endocytosis of the Notch ligand Delta, and loss of faf or lqf activity results in perturbed Notch signaling and defective eye development. In addition to its functions in endocytosis, the human Faf homolog, Usp9, affects progression through mitosis by deubiquitinating the chromosomal passenger protein survivin [75]. By controlling the endocytosis and lysosomal trafficking of activated growth- and differentiation factor receptors, DUBs form a critical link between proliferation and cell cycle exit.

Regulation of cell cycle checkpoints

It is vital for multicellular organisms to prevent mistakes in proliferation. This is the task of checkpoint signaling networks that sense intracellular damage or incomplete cell cycle stages, and as a consequence, reversibly stall cell cycle progression. Following the repair of damage or completion of a cell cycle stage, the checkpoints are silenced and the cell cycle machinery resumes. Two well studied examples are the DNA damage checkpoint and the mitotic spindle checkpoint, both of which are intimately controlled by deubiquitination and impaired in a wide range of cancers.

The DNA damage checkpoint senses different kinds of DNA damage, such as DNA crosslinks or breaks, leading to activation of kinase cascades and cell cycle arrest in G1 or G2. A key component of the DNA damage checkpoint is the transcription factor p53. Its regulation by opposing activities, Mdm2-dependent ubiquitination/degradation and Usp7/Hausp-dependent deubiquitination/stabilization, was described before. Similar to Usp7/Hausp, Usp28 protects crucial DNA damage checkpoint components from unscheduled degradation [50]. Usp28 was originally isolated as a binding partner of the checkpoint mediators 53BP1, claspin, and Mdc1. The depletion of Usp28 by siRNA or expression of catalytically inactive Usp28 in a cell culture model triggered the premature degradation of its binding partners, but also of kinase specificity factors and an effector kinase, Chk2. This attenuates DNA damage signaling and interferes with p53-dependent apoptosis. As mentioned before, Usp28 also protects the transcription factor c-Myc from SCF^{Fbw7}-dependent ubiquitination, but dissociates from c-Myc upon DNA damage [51]. By controlling the turnover of checkpoint components and a proliferative transcription factor, Usp28 can elegantly co-regulate checkpoint activation and cell cycle progression. While Usp28 protects proteins from unwanted degradation, Usp1 regulates checkpoints non-proteolytically by counteracting the ubiquitination of FANCD2 and PCNA [27,76]. FANCD2 is transiently monoubiquitinated in S phase, which is required for normal cell cycle progression. Monoubiquitination also targets FANCD2 to sites of DNA breaks, where it interacts with recombination enzymes and participates in DNA repair. Similarly, monoubiquitination of PCNA occurs in response to DNA damage or stalled replication forks and recruits DNA-repair polymerases [77]. The Usp1/UAF1-complex directly binds to

modified FANCD2 and PCNA and catalyzes their deubiquitination [25]. In the event of DNA damage, Usp1 is inactivated by transcriptional repression, proteasomal degradation, and autocleavage. This triggers monoubiquitination of FANCD2 or PCNA and allows cells to initiate necessary DNA repair. The ubiquitination of FANCD2 is misregulated in patients with Fanconi Anemia, underscoring its importance for cell cycle regulation and maintaining genomic stability.

Upon entering mitosis, condensed chromosomes have to achieve bipolar attachment to the mitotic spindle, or otherwise cell division would result in aneuploid progeny. The genomic integrity of dividing cells is ensured by the spindle checkpoint, which is activated by mis- or unattached chromosomes [78]. As a consequence, the spindle checkpoint inhibits the critical ubiquitin ligase APC/C, which arrests cells at metaphase and gives them the necessary time to complete chromosome attachment. Once this is achieved, the spindle checkpoint is rapidly turned off and sister chromatid separation is initiated. Intriguingly, spindle checkpoint silencing is triggered by the APC/C-dependent multiubiquitination of the substrate-targeting factor Cdc20 and the resulting dissociation of its inhibitor Mad2 [79,80]. The DUB Usp44 counteracts APC/C-activation by deubiquitinating Cdc20, thereby preventing premature Mad2-dissociation and spindle checkpoint silencing [20; Figure 2]. Reducing the levels of Usp44 results in an almost complete loss of spindle checkpoint function. These phenotypes can be rescued by co-depleting APC/C-subunits or the APC/C-specific E2 UbcH10, which strongly suggests that Usp44 reinforces the spindle checkpoint by directly opposing APC/C-dependent ubiquitination.

Following the silencing of the spindle checkpoint, the fully activated APC/C orchestrates progression through mitosis by targeting several key mitotic regulators for sequential degradation [81]. This “substrate ordering” of the APC/C is also controlled by DUB activity, although the molecular nature of the respective enzymes remains unknown [82]. In this case, deubiquitination delays the assembly of ubiquitin chains on substrates, which bind the APC/C weakly and consequently are degraded late in mitosis. Deubiquitination, therefore, turns out to be a major regulator of the APC/C and progression through mitosis.

Outlook

The surge in recent studies on the reversible nature of ubiquitination has brought deubiquitination into the limelight of cell cycle regulation. DUBs also function in cell survival and development, and thus, it is not surprising that they are frequently mutated or misregulated in cancer. Our increasing understanding of their catalytic activity, regulation, and substrate specificity will empower us to exploit DUB inhibition as a therapeutic strategy against cancer. Indeed, initial studies support the idea that DUB inhibition is of therapeutic value [83]. Continuing our efforts to dissect DUB function and regulation will not only deepen our understanding of the complexities of cell cycle regulation, but it will almost certainly increase our chances of developing specific and effective anti-proliferative drugs.

Acknowledgements

We thank Lingyan Jin, Adam Williamson, and Julia Schaletzky for many fruitful discussions. We are also grateful to Julia Schaletzky for critically reading the manuscript. Our work is supported by a PEW Scholar Award and an NIH Director's New Innovator Award.

References

1. Kerscher O, Felberbaum R, Hochstrasser M. Modification of proteins by ubiquitin and ubiquitin-like proteins. *Annu Rev Cell Dev Biol* 2006;22:159–180. [PubMed: 16753028]

2. Potapova TA, Daum JR, Pittman BD, Hudson JR, Jones TN, Satinover DL, Stukenberg PT, Gorbisky GJ. The reversibility of mitotic exit in vertebrate cells. *Nature* 2006;440:954–958. [PubMed: 16612388]
3. Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, Sixma TK, Bernards R. A genomic and functional inventory of deubiquitinating enzymes. *Cell* 2005;123:773–786. [PubMed: 16325574]
- 4**. Hu M, Li P, Li M, Li W, Yao T, Wu JW, Gu W, Cohen RE, Shi Y. Crystal structure of a UBP-family deubiquitinating enzyme in isolation and in complex with ubiquitin aldehyde. *Cell* 2002;111:1041–1054. [PubMed: 12507430] This study elucidated the mechanism of ubiquitin binding and showed the important structural rearrangements occurring after ubiquitin recognition.
5. Johnston SC, Riddle SM, Cohen RE, Hill CP. Structural basis for the specificity of ubiquitin C-terminal hydrolases. *EMBO J* 1999;18:3877–3887. [PubMed: 10406793]
6. Iyer LM, Koonin EV, Aravind L. Novel predicted peptidases with a potential role in the ubiquitin signaling pathway. *Cell Cycle* 2004;3:1440–1450. [PubMed: 15483401]
7. Mao Y, Senic-Matuglia F, Di Fiore PP, Polo S, Hodsdon ME, De Camilli P. Deubiquitinating function of ataxin-3: insights from the solution structure of the Josephin domain. *Proc Natl Acad Sci USA* 2005;102:12700–12705. [PubMed: 16118278]
8. Yao T, Cohen RE. A cryptic protease couples deubiquitination and degradation by the proteasome. *Nature* 2002;419:403–407. [PubMed: 12353037]
9. Ambroggio XI, Rees DC, Deshaies RJ. JAMM: a metalloprotease-like zinc site in the proteasome and signalosome. *PLoS Biol* 2004;2:E2. [PubMed: 14737182]
10. Kattenhorn LM, Korbel GA, Kessler BM, Spooner E, Ploegh HL. A deubiquitinating enzyme encoded by HSV-1 belongs to a family of cysteine proteases that is conserved across the family Herpesviridae. *Mol Cell* 2005;19:547–557. [PubMed: 16109378]
11. Schlieker C, Korbel GA, Kattenhorn LM, Ploegh HL. A deubiquitinating activity is conserved in the large tegument protein of the herpesviridae. *J Virol* 2005;79:15582–15585. [PubMed: 16306630]
- 12*. Ova H. Active-site directed probes to report enzymatic action in the ubiquitin proteasome system. *Nature Rev Cancer* 2007;7:613–620. [PubMed: 17646866] Reviews innovative approaches for identifying DUBs involved in cellular regulation.
13. Schlieker C, Weihofen WA, Frijns E, Kattenhorn LM, Gaudet R, Ploegh HL. Structure of a herpesvirus-encoded cysteine protease reveals a unique class of deubiquitinating enzymes. *Mol Cell* 2007;25:677–687. [PubMed: 17349955]
14. Renatus M, Parrado SG, D'Arcy A, Eidhoff U, Gerhartz B, Hassiepen U, Pierrat B, Riedl R, Vinzenz D, Worpenberg S, Kroemer M. Structural basis of ubiquitin recognition by the deubiquitinating protease USP2. *Structure* 2006;14:1293–1302. [PubMed: 16905103]
15. Reyes-Turcu FE, Horton JR, Mullally JE, Heroux A, Cheng X, Wilkinson KD. The ubiquitin binding domain ZnF UBP recognizes the C-terminal diglycine motif of unanchored ubiquitin. *Cell* 2006;124:1197–1208. [PubMed: 16564012]
16. Sheng Y, Saridakis V, Sarkari F, Duan S, Wu T, Arrowsmith CH, Frappier L. Molecular recognition of p53 and MDM2 by USP7/HAUSP. *Nature Struct Mol Biol* 2006;13:285–291. [PubMed: 16474402]
17. Saito K, Kigawa T, Koshiba S, Sato K, Matsuo Y, Sakamoto A, Takagi T, Shirouzu M, Yabuki T, Nunokawa E, Seki E, Matsuda T, Aoki M, Miyata Y, Hirakawa N, Inoue M, Terada T, Nagase T, Kikuno R, Nakayama M, Ohara O, Tanaka A, Yokoyama S. The CAP-Gly domain of CYLD associates with the proline-rich sequence in NEMO/IKKgamma. *Structure* 2004;12:1719–1728. [PubMed: 15341735]
18. Verma R, Aravind L, Oania R, McDonald WH, Yates JR 3rd, Koonin EV, Deshaies RJ. Role of Rpn11 metalloprotease in deubiquitination and degradation by the 26S proteasome. *Science* 2002;298:611–615. [PubMed: 12183636]
19. Stegmeier F, Sowa ME, Nelapa G, Gygi SP, Harper JW, Elledge SJ. The tumor suppressor CYLD regulates entry into mitosis. *Proc Natl Acad Sci USA* 2007;104:8869–8874. [PubMed: 17495026]
- 20*. Stegmeier F, Rape M, Draviam VM, Nalepa G, Sowa ME, Ang XL, McDonald ER 3rd, Li MZ, Hannon GJ, Sorger PK, Kirschner MW, Harper JW, Elledge SJ. Anaphase initiation is regulated by antagonistic ubiquitination and deubiquitination activities. *Nature* 2007;446:876–881. [PubMed: 17495026]

- 17443180] This study describes the dynamic regulation of the spindle checkpoint by opposing ubiquitination and deubiquitination.
- 21*. Leggett DS, Hanna J, Borodovsky A, Crosas B, Schmidt M, Baker RT, Walz T, Ploegh H, Finley D. Multiple associated proteins regulate proteasome structure and function. *Mol Cell* 2002;10:495–507. [PubMed: 12408819] Demonstrates the activation of a deubiquitinating enzyme by interaction with non-catalytic subunits of the 26S proteasome.
 22. Hu M, Li P, Song L, Jeffrey PD, Chenova TA, Wilkinson KD, Cohen RE, Shi Y. Structure and mechanisms of the proteasome-associated deubiquitinating enzyme USP14. *EMBO J* 2005;24:3747–3756. [PubMed: 16211010]
 23. Yao T, Song L, Xu W, DeMartino GN, Florens L, Swanson SK, Washburn MP, Conaway RC, Conaway JW, Cohen RE. *Nature Cell Biol* 2006;8:994–1002. [PubMed: 16906146]
 24. Qiu XB, Ouyang SY, Li CJ, Miao S, Wang L, Goldberg AL. hRpn13/ADRM1/GP110 is a novel proteasome subunit that binds the deubiquitinating enzyme, UCH37. *EMBO J* 2006;25:5742–5753. [PubMed: 17139257]
 - 25*. Cohn MA, Kowal P, Yang K, Haas W, Huang TT, Gygi SP, D'Andrea AD. A UAF1-containing multisubunit protein complex regulates the Fanconi Anemia pathway. *Mol Cell* 2007;28:786–797. [PubMed: 18082604] This paper impressively shows the activation of a deubiquitinating enzyme by incorporation into specific protein complexes.
 26. Mizuno E, Kitamura N, Komada M. 14-3-3-dependent inhibition of the deubiquitinating activity of UBPY and its cancellation in the M phase. *Exp Cell Res* 2007;313:3624–3634. [PubMed: 17720156]
 - 27*. Huang TT, Nijman SMB, Mirchandani KD, Galardy PJ, Cohn MA, Haas W, Gygi SP, Ploegh HL, Bernards R, D'Andrea AD. Regulation of monoubiquitinated PCNA by DUB autocleavage. *Nature Cell Biol* 2006;8:339–347. [PubMed: 16531995] Introduces DUB-autocleavage as a novel mechanism of DUB regulation.
 28. Ryu KY, Maehr R, Gilchrist CA, Long MA, Bouley DM, Mueller B, Ploegh HL, Kopito RR. The mouse polyubiquitin gene UbC is essential for fetal liver development, cell-cycle progression and stress tolerance. *EMBO J* 2007;26:2693–2706. [PubMed: 17491588]
 29. Haas AL, Bright PM. The dynamics of ubiquitin pools within cultured human lung fibroblasts. *J Biol Chem* 1987;262:345–351. [PubMed: 3025210]
 30. Hanna J, Leggett DS, Finley D. Ubiquitin depletion as a key mediator of toxicity by translational inhibitors. *Mol Cell Biol* 2003;23:9251–9261. [PubMed: 14645527]
 31. Wilson SM, Bhattacharyya B, Rachel RA, Coppola V, Tessarollo L, Householder DB, Fletcher CF, Miller RJ, Copeland NG, Jenkins NA. Synaptic defects in ataxia mice result from a mutation in *Usp14*, encoding a ubiquitin-specific protease. *Nat Genetics* 2002;32:420–425. [PubMed: 12368914]
 32. Everett RD, Meredith M, Orr A, Cross A, Kathoria M, Parkinson J. A novel ubiquitin-specific protease is dynamically associated with the PML nuclear domain and binds to a herpesvirus regulatory protein. *EMBO J* 1997;16:1519–1530. [PubMed: 9130697]
 33. Li M, Chen D, Shiloh A, Luo J, Nikolaev AY, Qin J, Gu W. Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. *Nature* 2002;416:648–653. [PubMed: 11923872]
 - 34*. Li M, Brooks CL, Kon N, Gu W. A dynamic role of HAUSP in the p53-Mdm2 pathway. *Mol Cell* 2004;13:879–886. [PubMed: 15053880] This paper and reference 35 show convincing evidence for the direct regulation of ubiquitin ligase stability by deubiquitinating enzymes.
 35. Meulmeester E, Maurice MM, Boutell C, Teunisse AFAS, Ovaa H, Abraham TE, Dirks RW, Jochemsen AG. Loss of HAUSP-mediated deubiquitination contributes to DNA damage-induced destabilization of Hdmx and Hdm2. *Mol Cell* 2005;18:565–576. [PubMed: 15916963]
 36. Lahav G, Rosenfeld N, Sigal A, Geva-Zatorsky N, Levine AJ, Elowitz MB, Alon U. Dynamics of the p53-Mdm2 feedback loop in individual cells. *Nat Genetics* 2003;36:147–150. [PubMed: 14730303]
 37. Jensen DE, Proctor M, Marquis ST, Gardner HP, Ha SI, Chodosh LA, Ishov AM, Tommerup N, Vissing H, Sekido Y, Minna J, Borodovsky A, Schultz DC, Wilkinson KD, Maul GG, Barlev N, Berger SL, Prendergast GC, Rauscher FJ 3rd. BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances BRCA1-mediated cell growth suppression. *Oncogene* 1999;16:1097–1112. [PubMed: 9528852]

38. Dong Y, Hakimi MA, Chen X, Kumaraswamy E, Cooch NS, Godwin AK, Shiekhatter R. Regulation of BRCC, a holoenzyme complex containing BRCA1 and BRCA2, by a signalosome-like subunit and its role in DNA repair. *Mol Cell* 2003;12:1087–1099. [PubMed: 14636569]
39. Mallery DL, Vandenberg CJ, Hiom K. Activation of the E3 ligase function of the BRCA1/BARD1 complex by polyubiquitin chains. *EMBO J* 2002;21:6755–6762. [PubMed: 12485996]
- 40**. Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, Wu P, Wiesmann C, Baker R, Boone DL, Ma A, Koonin EV, Dixit VM. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. *Nature* 2004;430:694–699. [PubMed: 15258597] This is the most striking example to date of co-regulation of ubiquitination and deubiquitination – both activities are found within the same polypeptide chain.
41. Chen ZJ. Ubiquitin signalling in the NF-kappaB pathway. *Nat Cell Biol* 2005;7:758–765. [PubMed: 16056267]
- 42**. Brummelkamp TR, Nijman SMB, Dirac AMG, Bernards R. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kB. *Nature* 2003;424:797–801. [PubMed: 12917690] This manuscript describes one of the first successful siRNA-screens for DUB-targets, which together with concomitant publications implied a therapeutic strategy to treat familial cylindromatosis.
43. Massoumi R, Chmielarska K, Hennecke K, Pfeifer A, Fässler R. Cyld inhibits tumor cell proliferation by blocking Bcl-3-dependent NF-kappaB signaling. *Cell* 2006;125:665–677. [PubMed: 16713561]
44. Kovalenko A, Chable-Bessia C, Cantarella G, Israël A, Wallach D, Courtois G. The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. *Nature* 2003;424:801–805. [PubMed: 12917691]
45. Salghetti SE, Muratani M, Wijnen H, Futcher B, Tansey WP. Functional overlap of sequences that activate transcription and signal ubiquitin-mediated proteolysis. *Proc Natl Acad Sci USA* 2000;97:3118–3123. [PubMed: 10706616]
46. Welcker M, Orian A, Grim JE, Eisenman RN, Clurman BE. A nucleolar isoform of the Fbw7 ubiquitin ligase regulates c-Myc and cell size. *Curr Biol* 2004;14:1852–1857. [PubMed: 15498494]
47. Kim SY, Herbst A, Tworkowski KA, Salghetti SE, Tansey WP. Skp2 regulates myc protein stability and activity. *Mol Cell* 2003;11:1189–1200. [PubMed: 12769844]
48. Strohmaier H, Spruck CH, Kaiser P, Won KA, Sangfelt O, Reed SI. Human F-box protein hCdc4 targets cyclin E for proteolysis and is mutated in a breast cancer cell line. *Nature* 2001;413:316–322. [PubMed: 11565034]
- 49**. Popov N, Wanzel M, Madiredjo M, Zhang D, Beijersbergen R, Bernards R, Moll R, Elledge SJ, Eilers M. The ubiquitin-specific protease USP28 is required for MYC stability. *Nat Cell Biol* 2007;9:765–774. [PubMed: 17558397]
- 50**. Zhang D, Zaugg K, Mak TW, Elledge SJ. A role for the deubiquitinating enzyme USP28 in control of the DNA damage response. *Cell* 2006;126:529–542. [PubMed: 16901786] Both references 49 and 50 identify Usp28 as a cell cycle regulator that can couple DNA repair and proliferation.
51. Popov N, Herold S, Llamazares M, Schuelein C, Eilers M. Fbw7 and Usp28 regulate myc protein stability in response to DNA damage. *Cell Cycle* 2007;6:2327–2331. [PubMed: 17873522]
52. Gavioli R, Frisan T, Vertuani S, Bornkamm GW, Masucci MG. C-myc overexpression activates alternative pathways for intracellular proteolysis in lymphoma cells. *Nature Cell Biol* 2001;3:283–288. [PubMed: 11231578]
53. Rape M, Jentsch S. Productive RUPTure: activation of transcription factors by proteasomal processing. *Biochim Biophys Acta* 2004;1695:209–213. [PubMed: 15571816]
54. van der Horst A, de Vries-Smits AM, Brenkman AB, van Triest MH, van den Broek N, Colland F, Maurice MM, Burgering BM. FOXO4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP. *Nat Cell Biol* 2006;8:1064–1073. [PubMed: 16964248]
55. Hoeller D, Hecker CM, Dikic I. Ubiquitin and ubiquitin-like proteins in cancer pathogenesis. *Nat Rev Cancer* 2006;6:776–788. [PubMed: 16990855]
56. Sun ZW, Allis CD. Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. *Nature* 2002;418:104–108. [PubMed: 12077605]
57. Goldknopf IL, French MF, Musso R, Busch H. Presence of protein A24 in rat liver nucleosomes. *Proc Natl Acad Sci USA* 1977;74:5492–5495. [PubMed: 271971]

58. Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones RS, Zhang Y. Role of histone H2A ubiquitination in Polycomb silencing. *Nature* 2004;431:873–878. [PubMed: 15386022]
59. Wu RS, Kohn KW, Bonner WM. Metabolism of ubiquitinated histones. *J Biol Chem* 1981;256:5916–5920. [PubMed: 6263895]
60. Joo HY, Zhai L, Yang C, Nie S, Erdjument-Bromage H, Tempst P, Chang C, Wang H. Regulation of cell cycle progression and gene expression by H2A deubiquitination. *Nature* 2007;449:1068–1072. [PubMed: 17914355]
61. Nicassio F, Corrado N, Vissers JH, Areces LB, Bergink S, Marteijn JA, Geverts B, Houtsmuller AB, Vermeulen W, Di Fiore PP, Citterio E. Human USP3 is a chromatin modifier required for S phase progression and genome stability. *Curr Biol* 2007;17:1972–1977. [PubMed: 17980597]
62. Wyce A, Xiao T, Whelan KA, Kosman C, Walter W, Eick D, Hughes TR, Krogan NJ, Strahl BD, Berger SL. H2B ubiquitylation acts as a barrier to Ctk1 nucleosomal recruitment prior to removal by Ubp8 within a SAGA-related complex. *Mol Cell* 2007;27:275–288. [PubMed: 17643376]
63. Murphy LO, Smith S, Chen RH, Fingar DC, Blenis J. Molecular interpretation of ERK signal duration by immediate early gene products. *Nat Cell Biol* 2002;4:556–564. [PubMed: 12134156]
64. Huang F, Kirkpatrick D, Jiang X, Gygi S, Sorkin A. Differential regulation of EGF receptor internalization and degradation by multiubiquitination within the kinase domain. *Mol Cell* 2006;21:737–748. [PubMed: 16543144]
65. Mukhopadhyay D, Riezman H. Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science* 2007;315:201–205. [PubMed: 17218518]
66. Row PW, Prior IA, McCullough J, Clague MJ, Urbe S. The ubiquitin isopeptidase UBPY regulates endosomal ubiquitin dynamics and is essential for receptor downregulation. *J Biol Chem* 2006;281:12618–12624. [PubMed: 16520378]
67. Bowers K, Piper SC, Edeling MA, Gray SR, Owen DJ, Lehner PJ, Luzio JP. Degradation of endocytosed epidermal growth factor and virally ubiquitinated major histocompatibility complex class I is independent of mammalian ESCRTII. *J Biol Chem* 2006;281:5094–5105. [PubMed: 16371348]
68. Mizuno E, Iura T, Mukai A, Yoshimori T, Kitamura N, Komada M. Regulation of epidermal growth factor receptor down-regulation by UBPY-mediated deubiquitination at endosomes. *Mol Cell Biol* 2005;16:5163–5174.
69. Niendorf S, Oksche A, Kisser A, Loehler J, Prinz M, Schorle H, Feller S, Lewitzky M, Horak I, Knobeloch KP. Essential role of ubiquitin-specific protease 8 for receptor tyrosine kinase stability and endocytic trafficking in vivo. *Mol Cell Biol* 2007;27:5029–5039. [PubMed: 17452457]
70. Naviglio S, Matteucci C, Matoskova B, Nagase T, Nomura N, Di Fiore PP, Draetta GF. UBPY: a growth-regulated human ubiquitin isopeptidase. *EMBO J* 1998;17:3241–3250. [PubMed: 9628861]
71. Janssen JW, Schleithoff L, Bartram CR, Schulz AS. An oncogenic fusion product of the phosphatidylinositol 3-kinase p85 β subunit and HUMORF8, a putative deubiquitinating enzyme. *Oncogene* 1998;16:1767–1772. [PubMed: 9582025]
72. Amerik A, Sindhi N, Hochstrasser M. A conserved late endosome-targeting signal required for Doa4 deubiquitylating enzyme function. *J Cell Biol* 2006;175:825–835. [PubMed: 17145966]
73. Overstreet E, Fitch E, Fischer JA. Fat facets and Liquid facets promote Delta endocytosis and Delta signaling in the signaling cells. *Development* 2004;131:5355–5366. [PubMed: 15469967]
74. McCullough J, Row PE, Lorenzo O, Doherty M, Beynon R, Clague MJ, Urbé S. Activation of the endosome-associated ubiquitin isopeptidase AMSH by STAM, a component of the multivesicular body-sorting machinery. *Curr Biol* 2006;16:160–165. [PubMed: 16431367]
75. Vong QP, Cao K, Li HY, Iglesias PA, Zheng Y. Chromosome alignment and segregation regulated by ubiquitination of survivin. *Science* 2005;310:1499–1504. [PubMed: 16322459]
76. Nijman SMB, Huang TT, Dirac AMG, Brummelkamp TR, Kerkhoven RM, D'Andrea AD, Bernards R. The deubiquitinating enzyme USP1 regulates the Fanconi Anemia pathway. *Mol Cell* 2005;17:331–339. [PubMed: 15694335]
77. Moldovan GL, Pfander B, Jentsch S. PCNA, the maestro of the replication fork. *Cell* 2007;129:665–679. [PubMed: 17512402]
78. Musacchio A, Salmon ED. The spindle-assembly checkpoint in space and time. *Nat Rev Mol Cell Biol* 2007;8:379–393. [PubMed: 17426725]

79. Xia G, Luo X, Habu T, Rizo J, Matsumoto T, Yu H. Conformation-specific binding of p31(comet) antagonizes the function of Mad2 in the spindle checkpoint. *EMBO J* 2004;23:3133–3143. [PubMed: 15257285]
80. Reddy SK, Rape M, Margansky WA, Kirschner MW. Ubiquitination by the anaphase-promoting complex drives spindle checkpoint inactivation. *Nature* 2007;446:921–925. [PubMed: 17443186]
81. Peters JM. The anaphase promoting complex/cyclosome: a machine designed to destroy. *Nat Rev Mol Cell Biol* 2006;7:644–656. [PubMed: 16896351]
- 82*. Rape M, Reddy SK, Kirschner MW. The processivity of multiubiquitination by the APC determines the order of substrate degradation. *Cell* 2006;124:89–103. [PubMed: 16413484] This study shows how deubiquitination can regulate the timing of degradation of different substrates of the same ubiquitin ligase.
83. Liu Y, Lashuel HA, Choi S, Xing X, Case A, Ni J, Yeh LA, Cuny GD, Stein RL, Lansbury PT Jr. Discovery of inhibitors that elucidate the role of UCH-L1 activity in the H1299 lung cancer cell line. *Chem Biol* 2003;10:837–846. [PubMed: 14522054]
84. Rumpf S, Jentsch S. Functional division of substrate processing cofactors of the ubiquitin-selective Cdc48 chaperone. *Mol Cell* 2006;21:261–269. [PubMed: 16427015]

Figure 1.

Usp1 as an example for multi-layered DUB regulation. Usp1 expression is regulated in a cell cycle-dependent manner, but newly synthesized, monomeric Usp1 has low activity. Oligomerization with a WD40-repeat containing binding partner, UAF1, stimulates the DUB-activity of Usp1. Usp1, however, can initiate its inactivation by catalyzing the cleavage of its own catalytic domain. Dissociation from UAF1 will complete Usp1 inactivation and potentially trigger its proteasomal degradation.

Figure 2.

Usp44 as an example of DUB-dependent cell cycle control. When the spindle checkpoint is active, the ubiquitin ligase APC/C is inhibited by binding of Mad2 to the substrate-targeting factor Cdc20. Following the completion of chromosome attachment, the p31comet protein is activated and allows APC/C and UbcH10 to catalyze multiubiquitination of Cdc20. The APC/C-dependent multiubiquitination triggers dissociation of the Mad2 inhibitor and full activation of the APC/C. Premature activation of the APC/C is inhibited by the Usp44 deubiquitinating enzyme. Usp44 is activated in mitosis, which potentially requires its mitotic phosphorylation or recruitment of binding partners. Inhibition of CDK-activity, which is caused by the APC/C-dependent degradation of cyclins, leads to degradation of Usp44. Thus, Usp44 both regulates the APC/C and is regulated, albeit indirectly, by the APC/C.