ELSEVIER

Otubains

DATABANKS

MEROPS name: Cezanne-2 peptidase
MEROPS classification: clan CA, family C64, peptidase C64.002
Species distribution: subphylum Vertebrata
Reference sequence from: *Homo sapiens* (UniProt: Q8TE49)

MEROPS name: A20 peptidase
MEROPS classification: clan CA, family C64, peptidase C64.003
Tertiary structure: Available

Species distribution: subphylum Vertebrata
Reference sequence from: *Homo sapiens* (UniProt: P21580)

MEROPS name: trabid peptidase
MEROPS classification: clan CA, family C64, peptidase C64.004
Species distribution: subkingdom Metazoa
Reference sequence from: *Homo sapiens* (UniProt: Q9UGI0)

MEROPS name: VCIP135 deubiquitinating peptidase
MEROPS classification: clan CA, family C64, peptidase C64.006

Species distribution: subphylum Vertebrata
Reference sequence from: *Homo sapiens* (UniProt: Q96JH7)

MEROPS name: otubain-1
MEROPS classification: clan CA, family C65, peptidase C65.001
Tertiary structure: Available
Species distribution: superkingdom Eukaryota
Reference sequence from: *Homo sapiens* (UniProt: Q96FW1)

MEROPS name: otubain-2
MEROPS classification: clan CA, family C65, peptidase C65.002
Tertiary structure: Available
Species distribution: subphylum Vertebrata
Reference sequence from: *Homo sapiens* (UniProt: Q96DC9)

MEROPS name: OTLD1 deubiquitinylating enzyme
MEROPS classification: clan CA, family C85, peptidase C85.001
Species distribution: superkingdom Eukaryota
Reference sequence from: *Homo sapiens* (UniProt: Q96G74)

MEROPS name: KIAA0459 (*Homo sapiens*)-like protein
MEROPS classification: clan CA, family C85, peptidase C85.003
Species distribution: superkingdom Eukaryota
Reference sequence from: *Homo sapiens*

MEROPS name: Otud1 protein
MEROPS classification: clan CA, family C85, peptidase C85.004
Species distribution: phylum Chordata
Reference sequence from: *Homo sapiens*

MEROPS name: YOD1 peptidase
MEROPS classification: clan CA, family C88, peptidase C88.002
Species distribution: superkingdom Eukaryota
Reference sequence from: *Homo sapiens* (UniProt: Q5VVQ6)

MEROPS name: OTU1 peptidase (*Saccharomyces cerevisiae*-type)
MEROPS classification: clan CA, family C88, peptidase C88.001
Tertiary structure: Available
Species distribution: order Saccharomycetales
Reference sequence from: *Saccharomyces cerevisiae* (UniProt: P43558)

MEROPS name: nairovirus deubiquitinylating peptidase
MEROPS classification: clan CA, family C87, peptidase C87.001

Tertiary structure: Available
Species distribution: known only from Crimean-Congo hemorrhagic fever virus
Reference sequence from: Crimean-Congo hemorrhagic fever virus

Name and History

Ubiquitylation is a reversible reaction, and removal of Ubiquitin (Ub) moieties is mediated by deubiquitylating enzymes (DUBs), which are predominantly enzymes of the cysteine- and metallo-protease families (see Chapters 351, 460−480). DUBs consist of four protease subclasses, the USPs (Chapters 460−462), UCHs (Chapter 463−470), Machado-Josephin domain (MJD) (Chapter 479) and ovarian tumor domain containing protease (OTU) families [1,2]. The latter is a group of proteins found primarily in viruses, plants, bacteria and eukaryotes. The eukaryotic sequences are originally related to the Ovarian Tumor (OTU) gene in *Drosophila*, cezanne deubiquitylating peptidase and tumor necrosis factor, alpha-induced protein 3 (*MEROPS* peptidase family C64) and otubain 1/otubain 2 (*MEROPS* peptidase family C65) in mammals (Table 477.1 and *MEROPS* Database).

OTU containing proteins have a sequence encoding a cysteine protease signature that is conserved across plants, viruses to higher eukaryotes. Approximately 100 genes are known to encode OTU domains, and research in recent year has shed light on the biological function of a considerable number of these enzymes establishing their role as Ubiquitin and Ubiquitin-like (Ubl) processing/deubiquitylating enzymes (DUBs). Structural studies of mammalian and viral OTUs reveal a substantial variety in Ubiquitin binding modes, and viral OTUs appear to have acquired the ability to also recognize ISG-15. OTUs play a role in cell biological processes such as ERAD (YOD1), but also immune signaling (Cezanne, DUBA, and OTUB1) and host-pathogen interactions (OTUB1/OTUB2, vOTUs). In this chapter, most recent advances in characterizing the biological functions of members of this DUB enzyme family are discussed.

Activity and Specificity

Sequence comparison bioinformatics revealed an evolutionary conserved domain encoding a cysteine protease motif [3]. This motif was first characterized in a gene that is involved in the development of the *Drosophila melanogaster* ovary (termed *otu* gene) where it may regulate the localization and translation of certain RNA transcripts [4,5]. Using the *Drosophila otu* gene and its homologs as a starting point, sequence similarities were found between these genes and those encoding viral and plant cysteine proteases.

TABLE 477.1 Examples[*] of Ovarian Tumor Domain (OTU) containing proteases with DUB-like activity

Name(s)	Origin	UniprotKB[a]	MEROPS	Biological function/Substrates	References
OTU1 (YOD1, OTUD2, HIN7)	human	Q5VVQ6	C88	DUB in ERAD	[37]
OTUD1 (DUBA-7)	human	Q5VV17 C85		unknown	UniprotKB
OTUB1 (HSPC263)	human	Q96FW1	C65	DUB stabilizing RNF128, RhoA, ERα, inh. RNF168, TRAF3/6	[38−41,44]
OTUB2	human	Q96DC9	C65	unknown	[10]
OTUD3	human	Q5T2D3		unknown	UniprotKB
OTUD4/HIN1L	human	Q01804	C85	unknown	UniprotKB
OTUD5 (DUBA)	human	Q96G74	C85	TRAF3 deubiquitylation	[29]
OTUD6A (DUBA2)/6B	human	Q7L8S5		unknown	UniprotKB
OTUD6B (DUBA5)	human	Q8N6M0		unknown	UniprotKB
OTUD7 (Cezanne 2)	human	Q8TE49	C64	DUB-like activity	UniprotKB
VCPIP1 (VCIP135)	human	Q96JH7	C64	DUB in ER, Golgi	[34,35]
TRABID (ZRANB1)	human	Q9UGI0	C64	Wnt signaling	[32]
A20 (OTUD7C)	human	P21580	C64	inflammatory signaling Pathways	[20,21]
OTU1 (yOTU1)	yeast	P43558	C88	interacts with p97/cdc48	[50]
L_CCHFI	virus	Q6TQR6	C87	de-Ub/ISGylation	[47]
OTLD1	plant	AT2G27350[b]	C85	histone DUB	[51]

[*]This list mentions the most commonly characterized proteases containing an OTU domain (in bold). More than ∼100 genes containing a conserved OTU domain in eukaryotes, prokaryotes and viruses are predicted to have cysteine protease activity.
[a]Accession numbers derived from SwissProt.
[b]Accession number derived from UniGene/NCBI.

First functional evidence for general DUB-like activity of OTU domain containing proteins, as demonstrated for OTUB1 (HSPC263), was provided by a chemical proteomics screen using Ubiquitin-based active site probes containing C-terminal elecrophilic groups acting as suicide inhibitors [6]. In subsequent studies, Otubain-1 and Otubain-2 were the first two OTU proteins found to display *in vitro* DUB activity [7]. Shortly thereafter, Cezanne, another OTU-domain containing protein, was found to interact with poly-Ub in a yeast two-hybrid assay and to contain DUB activity *in vitro*, suggesting that this is a general OTU feature [8]. Since the discovery of the first ovarian tumor domain (OTU) protease in *Drosophila* oogenesis and prior to the identification of vOTU, OTU superfamily members could be divided into two subclasses according to their sequence homology, otubains (*MEROPS* peptidase family C65) and A20-like OTUs including ZRANB1 (*MEROPS* peptidase family C64). With the addition of the viral OTU subclass, OTU superfamily members count more than 100 eukaryotic, bacterial, and viral proteins have now been identified (for examples, see Table 477.1). Predominantly, OTU proteases have been linked to Ubiquitin (Ub) and Ubiquitin-like protein (Ubl) removal and/or remodeling of Ub/Ubl-conjugated proteins, placing them among the protease superfamilies that facilitate signal transduction cascades and play key roles in protein stability [9].

Structural Chemistry

Sequence comparison of the catalytic regions of OTUs reveals a conserved Cys and His residues (Figure 477.1). However, the position of the acidic residue(s) is variable, and the amino acid side chains involved in the hydrogen bonding network responsible for rendering the catalytic thiol more nucleophilic are not always clearly evident from the primary sequence. In attempts to better understand the biological function of proteins belonging to the OTU superfamily, structures of several OTUs and OTU domains have been elucidated. The crystal structure of human OTUB2 confirmed the typical papain-like cysteine protease fold of the OTU domain (Figure 477.2A, left panel) and shows that, unlike other cysteine protease DUBs, the catalytic triad is incomplete and is stabilized by a new method involving a hydrogen bonding network (Figure 477.2A, right panel) [10]. Subsequent X-ray
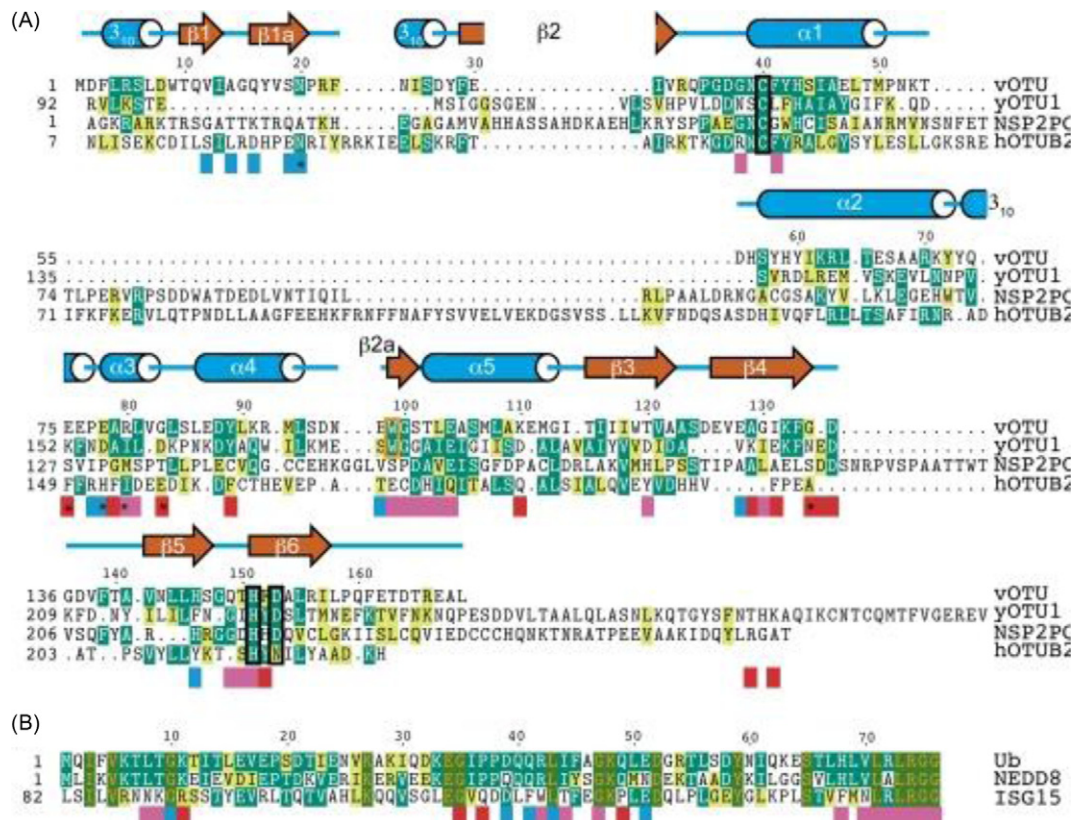
FIGURE 477.1 Sequence alignment of several OTU proteases. (A) OTUs are from CCHF virus (vOTU; Sprot accession no. Q6TFZ7), *Saccharomyces cerevisiae* (yOTU1; PDB accession no. 3BY4_A), PRRSV (NSP2PC; Sprot accession no. C1K780), and *Homo sapiens* Otubain2 (PDB accession no. 1TFF_A). Secondary structure of vOTU according to the defined secondary structure of proteins (DSSP) algorithm is represented by blue cylinders (helical regions), brown arrows (β-sheet regions), and blue lines (loops). Breaks denote regions where vOTU has residues. Residues involved in vOTU's catalytic triad are outlined in black boxes. Residues outlined in orange boxes represent those involved in oxyanion hole formation. Colored bars below the sequence alignment indicate vOTU and yOTU1 positions within 4 Å of Ub. Bars under vOTU unique positions are colored light blue with bars under unique yOTU1 positions in red. Equivalent positions in both vOTU and yOTU1 are shown in purple. Positions that form significant salt bridge and H-bond interactions are highlighted by an asterisk; (B) Ub and Ub-like proteins from *H. sapiens* (PDB accession no. 1UBQ_A), NEDD8 (Sprot accession no. Q15843), and ISG15 (PDB accession no. 1Z2M_A). Colored bars below the sequence alignment indicate Ub positions within 4Å of vOTU and yOTU1. Bars colored light blue and red indicate Ub positions that interact solely with vOTU and yOTU1, respectively. Purple bars indicate Ub residues within 4Å of both vOTU and yOTU1. Amino acids are color coded according to their being non-conserved (white background), similar (lime green background), conserved (green background), or completely conserved (dark-green background, orange lettering) across the four sequences (with courtesy of Capodagli *et al.* [16]).

structures of the human A20 OTU domain and OTUB1 indicated that the catalytic site is in an unproductive conformation in the apo enzyme as observed for other DUBs (Figure 477.2B and Johnston *et al.* [11], Hu *et al.* [12], and Edelmann *et al.* [13]). The binding of Ubiquitin leads to a conformational change that rearranges the catalytic site conformation as demonstrated in the crystal structure of yeast ovarian tumor 1 (yOTU1) in complex with mono-Ub [14]. Recently, the crystal structures of viral OTUs in complex with either Ubiquitin or ISG15 provided some insights into the molecular basis for the specificity and cross-reactivity of recognizing Ubiquitin and ISG15. As demonstrated for the structure of Crimean-Congo haemorrhagic fever virus (CCHFV) bound to Ubiquitin, the binding mode resembles the one observed

for other mammalian DUBs with Ubiquitin [15−17]. Interestingly, a comparison with the X-ray structure of CCHFV OTU in complex with ISG15 revealed that ISG15 is bound in an orientation 75° as to the one observed with Ubiquitin [17], thereby exposing different surfaces of the Ub/Ubl substrate to the enzyme [15]. The promiscuity of the CCHFV deubiquitylase towards different Ub/Ubls may represent a common phenomenon for vOTUs as well as other pathogen encoded DUBs, as this is also observed in the severe acute respiratory syndrome (SARS) corona virus encoded DUB PLpro [18] (Chapter 480). The fact that mammalian OTUs appear not to show cross-reactivity towards interferon induced ISG15, but other Ubls such as NEDD8 [13] may demonstrate that vOTUs underwent a co-evolutionary process provoked by
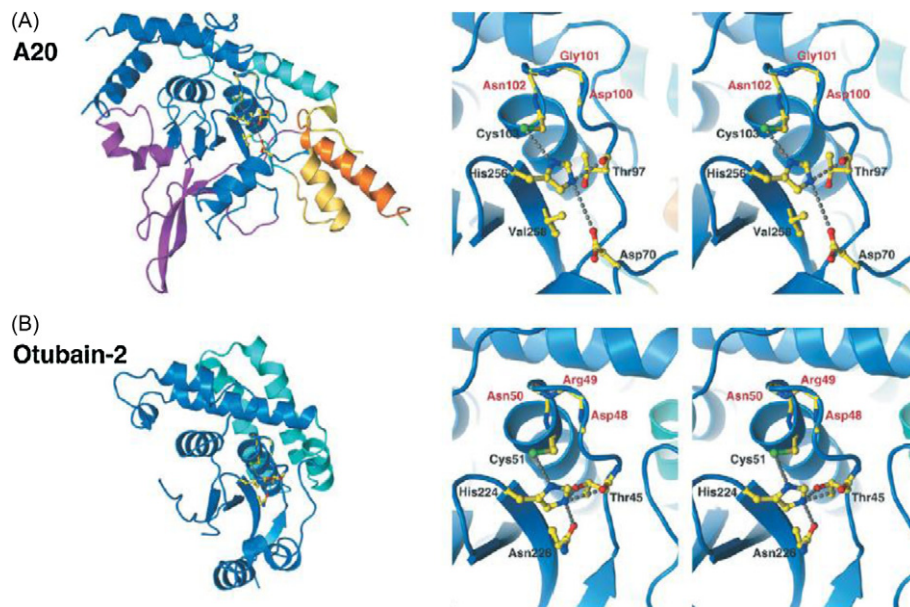
FIGURE 477.2 A20 and OTUB2 crystal structures reveal a conserved papain-like cysteine protease fold within the ovarian tumor domain but unique catalytic center configurations. (A) Ribbon structure of A20 (left panel) and stereo view of the catalytic center in which the catalytic residues and the relevant hydrogen bonds are highlighted (right panel); (B) Otubain-2 (OTUB2) ribbon structure (left panel and stereo view of the catalytic center in which the catalytic residues and the relevant hydrogen bonds are highlighted (right panel) (with courtesy of Komander & Barford [52]).

host-pathogen interactions. It remains to be seen whether any of the vOTUs may also show reactivity to the other interferon-induced Ubl FAT10, and whether the unique structural properties of vOTUs can be exploited for pharmacological inhibition based on small-molecule strategies as novel antiviral agents.

Preparation

Mammalian and viral OTU genes have been cloned and expressed as recombinant enzymes in bacterial expression systems. Some of these constructs are available commercially (ENZO Life Sciences, Life Sensors, Boston Biochem), but the majority of them are only available through academic research groups.

Biological Aspects

A20 and Inflammation

A20 was first characterized in human umbilical vein endothelial cells predominantly induced by the cytokines TNFα, IL-1β, and LPS [19]. A20 is also known as tumor necrosis factor-a-induced protein 3 (TNFAIP3). Analysis of the A20 domain revealed seven repeats of an A20-type zinc finger (ZnF-A20) in one single polypeptide chain, which exhibits E3 ligase activity, modulating the ubiquitylation status of key adaptors in the NF-κB signaling cascade [20,21]. The OTU domain that contains DUB protease activity is located at the N-terminus of A20. Overexpression of A20 inhibits TNF-mediated cell death and down-regulates NF-κB signaling. Upon treatment with TNF, A20 expression was found to increase in

variety of cells [20]. Mice deficient in A20 were prone to inflammation, and persistent activation of NF-κB by Toll-like and TNF receptors was observed in these mice [22]. Thus, A20 is critical for limiting inflammation by terminating TNF induced NF-κB responses *in vivo*. The constitutive expression of A20 in different cell types prevents TNFα-induced cell apoptosis. Furthermore, it has been reported that a loss of A20 expression increased the lethality of TNF-α and lipopolysaccharides due to activation of NF-κB [23]. The importance of A20 as a modulator of immunopathology is underpinned by the genetic association between several mutations in the human A20 locus and immunopathologies such as Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus, psoriasis and type 1 diabetes [24,25]. More recently, A20 was discovered to interact with the E3 ligase RNF11 and the E2 enzymes UBC13 Ubc5c, the latter of which antagonizes the activity of the E3 ligases TRAF6, TRAF2 and clAP1, thereby providing a direct mechanism of action of A20 terminating NF-κB activation [26,27]. Taken together, A20 represents an OTU with a central role in regulating inflammation, and mutations are associated with many autoimmune pathologies as well as B-cell malignancies, such as Hodgkin lymphomas [28].

Cezanne, TRABID, DUBA — Regulation of Immune Signaling Pathways

A number of other DUBs, in particular OTUs, have been found to also play a role in immune signaling pathways. Interferon type I (IFN-I) responses are triggered by pattern-recognition receptors (PRRs), and DUBA (OTUD5) was shown to negatively regulate this immune signaling

pathway [29]. DUBA directly binds tumor necrosis factor receptor-associate factor 3 (TRAF3) and cleaves its K63-linked polyubiquitin chains, and its expression is suppressed during IL-1R1 stimulation [30].

Two additional A20-like proteins have been identified and shown to play a role in immune signaling pathways. Cellular zinc finger anti-NF-κ B (Cezanne) and TRAF binding domain (TRABID) appear to interact with TRAF6 [31]. Cezanne has a negative effect on NF-κB activation, whereas TRABID activates Wnt-induced transcription [32]. These OTUs may also have other as yet undiscovered biological functions, as TRABID appears to have a preference of cleaving K29-linked over K63-linked polyubiquitin chains [33].

Roles of YOD1 and VCIP135 in ER Associated Protein Trafficking

A different subset of OTUs is involved in endoplasmic reticulum and Golgi associated biology. Initially, the AAA-ATPase p97/p47 complex was discovered to be required for heterotypic fusion of transport vesicles and assembly of mitotic Golgi fragments, and VCIP135 (VCIPI1) identified as an essential associated factor [34,35]. More recent studies on p97ATPase-mediated membrane fusion revealed that VCIP135 deubiquitylating activity is required for the p97/p47- but not the p97/p37-dependent pathway [36]. YOD1 (OTU1/OTUD2), another OTU family member, is directly implicated in the dislocation of misfolded proteins from the ER to the cytosol [37].

Pleiotropic Role of OTUB1

OTUB1 is abundantly expressed in most tissues [7] and was originally described as part of a complex including the E3 Ubiquitin ligase GRAIL (gene related to activation in lymphocytes) and USP8, and involved in regulating T-cell anergy [38]. However, OTUB1's ubiquituous expression profile predicts that it has additional biological roles not restricted to the lymphocytic lineage. Indeed, levels of oestrogen receptor α, TRAF3/6 and the small GTPase RhoA were suggested to be modulated by OTUB1 through direct deubiquitylation or indirect effects [39−41]. In addition, OTUB1 was found to regulate p53 stability [42] and bind UBC13 [43]. The latter negatively affects the Ubiquitin ligase RNF168 resulting in an inhibition of the DNA damage response [44]. The interaction with UBC13 and stabilization of p53 appear to be independent of its deubiquitylation activity (non-canonical effect), which may represent a different way that OTUs and perhaps other DUBs can function. The fact that OTUB1 binds UBC13 may have affects on other UBC13 dependent E3 ligases, which could explain its pleiotropic affect on many different (indirect) substrates. OTUB1 has DUB-like activity with an almost exclusive preference for K48-linked poly-Ubiquitin [7,13], suggesting that it may have DUB enzyme activity-dependent and independent functions.

Viral OTUs

The Ubiquitin-like protein ISG15 (Interferon stimulated gene 15) resembles a di-Ubiquitin moiety and mediates an antiviral response to certain viruses. Nairoviruses and Arteriviruses, two unrelated groups of RNA viruses, encode for ovarian tumor domain-containing sequences [45,46]. These viral OTUs appear to be expressed and to exert DUB-like activity as well as the ability to recognize and cleave ISG15, thereby inhibiting Ubiquitin and ISG15-dependent antiviral pathways [47]. For instance, for the porcine reproductive and respiratory syndrome (PRRS) virus, it was shown that the N-terminal part of the non-structural protein 2 (nsp2) inhibits NF-κB activation [48]. In addition, the Artevirus and Nairovirus OTUs target RIG-1-like receptors, a family of cytosolic RNA helicases that with Toll-like receptors (TLRs) belong to the family of pattern recognition receptors. In the case of Artevirus and Nairovirus derived OTUs, RIG-1 is deubiquitylated, thereby interfering with the signal induced by recognizing foreign RNA [49]. VOTUs are unique in that they are the only OTUs to have shown both deubiquitylating and deISGylating activity. In comparison, Otubain1/2 prefer K48-, K63-linked poly-Ub or NEDD8 (neural precursor cell expressed, developmentally downregulated 8) as substrates [7,13]. A20 and A20-like Cezanne OTU proteases selectively cleave K63-linked poly-Ub target and DUBA also shows preference for K63-linked poly-Ub [8,20,29]. This implies a considerable variation of the OTU domain to accommodate extended functionalities, in particular in viral genes to exploit the modulation of host-pathogen interactions.

Further Reading

The reader is directed to Reyes-Turcu *et al.* [1], Nijman *et al.* [2], Sowa *et al.* [43], and Frias-Staheli *et al.* [47].

References

[1] Reyes-Turcu, F.E., Ventii, K.H., Wilkinson, K.D. (2009). Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Annu. Rev. Biochem.* 78, 363−397.

[2] Nijman, S.M., Luna-Vargas, M.P., Velds, A., Brummelkamp, T.R., Dirac, A.M., Sixma, T.K., Bernards, R. (2005). A genomic and

functional inventory of deubiquitinating enzymes. *Cell* 123(5), 773−786.

[3] Makarova, K.S., Aravind, L., Koonin, E.V. (2000). A novel super-family of predicted cysteine proteases from eukaryotes, viruses and *Chlamydia pneumoniae*. *Trends Biochem. Sci.* 25(2), 50−52.

[4] Goodrich, J.S., Clouse, K.N., Schupbach, T. (2004). Hrb27C, Sqd and Otu cooperatively regulate gurken RNA localization and medi-ate nurse cell chromosome dispersion in *Drosophila oogenesis*. *Development* 131(9), 1949−1958.

[5] Steinhauer, W.R., Walsh, R.C., Kalfayan, L.J. (1989). Sequence and structure of the *Drosophila melanogaster* ovarian tumor gene and generation of an antibody specific for the ovarian tumor pro-tein. *Mol. Cell Biol.* 9(12), 5726−5732.

[6] Borodovsky, A., Ovaa, H., Kolli, N., Gan-Erdene, T., Wilkinson, K.D., Ploegh, H.L., Kessler, B.M. (2002). Chemistry-based functional proteomics reveals novel members of the deubi-quitinating enzyme family. *Chem. Biol.* 9(10), 1149−1159.

[7] Balakirev, M.Y., Tcherniuk, S.O., Jaquinod, M., Chroboczek, J. (2003). Otubains: a new family of cysteine proteases in the ubiqui-tin pathway. *EMBO Rep.* 4(5), 517−522.

[8] Evans, P.C., Smith, T.S., Lai, M.J., Williams, M.G., Burke, D.F., Heyninck, K., Kreike, M.M., Beyaert, R., Blundell, T.L., Kilshaw, P.J. (2003). A novel type of deubiquitinating enzyme. *J. Biol. Chem.* 278(25), 23180−23186.

[9] Katz, E.J., Isasa, M., Crosas, B. (2010). A new map to understand deubiquitination. *Biochem. Soc. Trans* 38(Pt 1), 21−28.

[10] Nanao, M.H., Tcherniuk, S.O., Chroboczek, J., Dideberg, O., Dessen, A., Balakirev, M.Y. (2004). Crystal structure of human otubain 2. *EMBO Rep.* 5(8), 783−788.

[11] Johnston, S.C., Larsen, C.N., Cook, W.J., Wilkinson, K.D., Hill, C.P (1997). Crystal structure of a deubiquitinating enzyme (human UCH-L3) at 1.8 Å resolution. *EMBO J.* 16(13), 3787−3796.

[12] Hu, M., Li, P., Song, L., Jeffrey, P.D., Chenova, T.A., Wilkinson, K.D., Cohen, R.E., Shi, Y. (2005). Structure and mechanisms of the proteasome-associated deubiquitinating enzyme USP14. *EMBO J.* 24(21), 3747−3756.

[13] Edelmann, M.J., Iphofer, A., Akutsu, M., Altun, M., di Gleria, K., Kramer, H.B., Fiebiger, E., Dhe-Paganon, S., Kessler, B.M. (2009). Structural basis and specificity of human otubain 1-medi-ated deubiquitination. *Biochem. J.* 418(2), 379−390.

[14] Messick, T.E., Russell, N.S., Iwata, A.J., Sarachan, K.L., Shiekhattar, R., Shanks, J.R., Reyes-Turcu, F.E., Wilkinson, K.D., Marmorstein, R. (2008). Structural basis for ubiquitin recognition by the Otu1 ovarian tumor domain protein. *J. Biol. Chem.* 283(16), 11038−11049.

[15] Akutsu, M., Ye, Y., Virdee, S., Chin, J.W., Komander, D. (2011). Molecular basis for ubiquitin and ISG15 cross-reactivity in viral ovarian tumor domains. *Proc. Natl. Acad. Sci. USA* 108(6), 2228−2233.

[16] Capodagli, G.C., McKercher, M.A., Baker, E.A., Masters, E.M., Brunzelle, J.S., Pegan, S.D. (2011). Structural analysis of a viral ovarian tumor domain protease from the Crimean-Congo hemor-rhagic fever virus in complex with covalently bonded ubiquitin. *J. Virol.* 85(7), 3621−3630.

[17] James, T.W., Frias-Staheli, N., Bacik, J.P., Levingston Macleod, J.M., Khajehpour, M., Garcia-Sastre, A., Mark, B.L.

(2011). Structural basis for the removal of ubiquitin and interferon-stimulated gene 15 by a viral ovarian tumor domain-containing protease. *Proc. Natl. Acad. Sci. USA* 108(6), 2222−2227.

[18] Lindner, H.A., Lytvyn, V., Qi, H., Lachance, P., Ziomek, E., Menard, R. (2007). Selectivity in ISG15 and ubiquitin recognition by the SARS coronavirus papain-like protease. *Arch. Biochem. Biophys.* 466(1), 8−14.

[19] Evans, P.C., Kilshaw, P.J. (2000). Interleukin-13 protects endothe-lial cells from apoptosis and activation: association with the protec-tive genes A20 and A1. *Transplantation* 70(6), 928−934.

[20] Evans, P.C., Ovaa, H., Hamon, M., Kilshaw, P.J., Hamm, S., Bauer, S., Ploegh, H.L., Smith, T.S. (2004). Zinc-finger protein A20, a regulator of inflammation and cell survival, has de-ubiquiti-nating activity. *Biochem. J.* 378(Pt 3), 727−734.

[21] Wertz, I.E., O'Rourke, K.M., Zhou, H., Eby, M., Aravind, L., Seshagiri, S., Wu, P., Wiesmann, C., Baker, R., Boone, D.L., Ma, A., Koonin, E.V., Dixit, V.M. (2004). De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-κB signalling. *Nature* 430(7000), 694−699.

[22] Boone, D.L., Turer, E.E., Lee, E.G., Ahmad, R.C., Wheeler, M.T., Tsui, C., Hurley, P., Chien, M., Chai, S., Hitotsumatsu, O., McNally, E., Pickart, C., Ma, A. (2004). The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. *Nat. Immunol.* 5(10), 1052−1060.

[23] Hitotsumatsu, O., Ahmad, R.C., Tavares, R., Wang, M., Philpott, D., Turer, E.E., Lee, B.L., Shiffin, N., Advincula, R., Malynn, B.A., Werts, C., Ma, A. (2008). The ubiquitin-editing enzyme A20 restricts nucleotide-binding oligomerization domain containing 2-triggered signals. *Immunity* 28(3), 381−390.

[24] Vereecke, L., Beyaert, R., van Loo, G. (2009). The ubiquitin-edit-ing enzyme A20 (TNFAIP3) is a central regulator of immunopa-thology. *Trends Immunol* 30(8), 383−391.

[25] Martin, F., Dixit, V.M. (2011). A20 edits ubiquitin and autoim-mune paradigms. *Nat. Genet.* 43(9), 822−823.

[26] Shembade, N., Parvatiyar, K., Harhaj, N.S., Harhaj, E.W. (2009). The ubiquitin-editing enzyme A20 requires RNF11 to downregu-late NF-κB signalling. *EMBO J.* 28(5), 513−522.

[27] Shembade, N., Ma, A., Harhaj, E.W. (2010). Inhibition of NF-kappaB signaling by A20 through disruption of ubiquitin enzyme complexes. *Science* 327(5969), 1135−1139.

[28] Malynn, B.A., Ma, A. (2009). A20 takes on tumors: tumor suppres-sion by an ubiquitin-editing enzyme. *J. Exp. Med.* 206(5), 977−980.

[29] Kayagaki, N., Phung, Q., Chan, S., Chaudhari, R., Quan, C., O'Rourke, K.M., Eby, M., Pietras, E., Cheng, G., Bazan, J.F., Zhang, Z., Arnott, D., Dixit, V.M. (2007). DUBA: a deubiquitinase that regulates type I interferon production. *Science* 318(5856), 1628−1632.

[30] Gonzalez-Navajas, J.M., Law, J., Nguyen, K.P., Bhargava, M., Corr, M.P., Varki, N., Eckmann, L., Hoffman, H.M., Lee, J., Raz, E. (2010). Interleukin 1 receptor signaling regulates DUBA expression and facilitates Toll-like receptor 9-driven antiinflamma-tory cytokine production. *J. Exp. Med.* 207(13), 2799−2807.

[31] Evans, P.C., Taylor, E.R., Coadwell, J., Heyninck, K., Beyaert, R., Kilshaw, P.J. (2001). Isolation and characterization of two novel A20-like proteins. *Biochem. J.* 357(Pt 3), 617−623.

[32] Tran, H., Hamada, F., Schwarz-Romond, T., Bienz, M. (2008). Trabid, a new positive regulator of Wnt-induced transcription with

preference for binding and cleaving K63-linked ubiquitin chains. *Genes Dev.* 22(4), 528−542.

[33] Virdee, S., Ye, Y., Nguyen, D.P., Komander, D., Chin, J.W. (2010). Engineered diubiquitin synthesis reveals Lys29-isopeptide specificity of an OTU deubiquitinase. *Nat. Chem. Biol.* 6(10), 750−757.

[34] Uchiyama, K., Jokitalo, E., Kano, F., Murata, M., Zhang, X., Canas, B., Newman, R., Rabouille, C., Pappin, D., Freemont, P., Kondo, H. (2002). VCIP135, a novel essential factor for p97/p47-mediated membrane fusion, is required for Golgi and ER assembly *in vivo*. *J. Cell Biol.* 159(5), 855−866.

[35] Wang, Y., Satoh, A., Warren, G., Meyer, H.H. (2004). VCIP135 acts as a deubiquitinating enzyme during p97-p47-mediated reassembly of mitotic Golgi fragments. *J. Cell Biol.* 164(7), 973−978.

[36] Totsukawa, G., Kaneko, Y., Uchiyama, K., Toh, H., Tamura, K., Kondo, H. (2011). VCIP135 deubiquitinase and its binding protein, WAC, in p97ATPase-mediated membrane fusion. *EMBO J.* 30(17), 3581−3593.

[37] Ernst, R., Mueller, B., Ploegh, H.L., Schlieker, C. (2009). The otubain YOD1 is a deubiquitinating enzyme that associates with p97 to facilitate protein dislocation from the ER. *Mol. Cell* 36(1), 28−38.

[38] Soares, L., Seroogy, C., Skrenta, H., Anandasabapathy, N., Lovelace, P., Chung, C.D., Engleman, E., Fathman, C.G. (2004). Two isoforms of otubain 1 regulate T cell anergy via GRAIL. *Nat. Immunol.* 5(1), 45−54.

[39] Stanisic, V., Malovannaya, A., Qin, J., Lonard, D.M., O'Malley, B.W. (2009). OTU Domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) deubiquitinates estrogen receptor (ER) alpha and affects ERalpha transcriptional activity. *J. Biol. Chem.* 284(24), 16135−16145.

[40] Edelmann, M.J., Kramer, H.B., Altun, M., Kessler, B.M. (2010). Post-translational modification of the deubiquitinating enzyme otubain 1 modulates active RhoA levels and susceptibility to *Yersinia* invasion. *FEBS J.* 277(11), 2515−2530.

[41] Li, S., Zheng, H., Mao, A.P., Zhong, B., Li, Y., Liu, Y., Gao, Y., Ran, Y., Tien, P., Shu, H.B. (2010). Regulation of virus-triggered signaling by OTUB1- and OTUB2-mediated deubiquitination of TRAF3 and TRAF6. *J. Biol. Chem.* 285(7), 4291−4297.

[42] Sun, X.X., Challagundla, K.B., Dai, M.S (2011). Positive regulation of p53 stability and activity by the deubiquitinating enzyme Otubain 1. *EMBO J.* 31, 576−592.

[43] Sowa, M.E., Bennett, E.J., Gygi, S.P., Harper, J.W. (2009). Defining the human deubiquitinating enzyme interaction landscape. *Cell* 138(2), 389−403.

[44] Nakada, S., Tai, I., Panier, S., Al-Hakim, A., Iemura, S., Juang, Y.C., O'Donnell, L., Kumakubo, A., Munro, M., Sicheri, F., Gingras, A.C., Natsume, T., Suda, T., Durocher, D. (2010). Non-canonical inhibition of DNA damage-dependent ubiquitination by OTUB1. *Nature* 466(7309), 941−946.

[45] Honig, J.E., Osborne, J.C., Nichol, S.T. (2004). Crimean-Congo hemorrhagic fever virus genome L RNA segment and encoded protein. *Virology* 321(1), 29−35.

[46] Kinsella, E., Martin, S.G., Grolla, A., Czub, M., Feldmann, H., Flick, R. (2004). Sequence determination of the Crimean-Congo hemorrhagic fever virus L segment. *Virology* 321(1), 23−28.

[47] Frias-Staheli, N., Giannakopoulos, N.V., Kikkert, M., Taylor, S.L., Bridgen, A., Paragas, J., Richt, J.A., Rowland, R.R., Schmaljohn, C.S., Lenschow, D.J., Snijder, E.J., García-Sastre, A., Virgin, H.W. IVth (2007). Ovarian tumor domain-containing viral proteases evade ubiquitin- and ISG15-dependent innate immune responses. *Cell Host Microbe* 2(6), 404−416.

[48] Sun, Z., Chen, Z., Lawson, S.R., Fang, Y. (2010). The cysteine protease domain of porcine reproductive and respiratory syndrome virus nonstructural protein 2 possesses deubiquitinating and interferon antagonism functions. *J. Virol.* 84(15), 7832−7846.

[49] van Kasteren, P.B., Beugeling, C., Ninaber, D.K., Frias-Staheli, N., van Boheemen, S., Garcia-Sastre, A., Snijder, E.J., Kikkert, M. (2012). Arterivirus and nairovirus ovarian tumor domain-containing Deubiquitinases target activated RIG-I to control innate immune signaling. *J. Virol.* 86(2), 773−785.

[50] Rumpf, S., Jentsch, S. (2006). Functional division of substrate processing cofactors of the ubiquitin-selective Cdc48 chaperone. *Mol. Cell* 21(2), 261−269.

[51] Krichevsky, A., Zaltsman, A., Lacroix, B., Citovsky, V. (2011). Involvement of KDM1C histone demethylase-OTLD1 otubain-like histone deubiquitinase complexes in plant gene repression. *Proc. Natl Acad. Sci. USA* 108(27), 11157−11162.

[52] Komander, D., Barford, D. (2008). Structure of the A20 OTU domain and mechanistic insights into deubiquitination. *Biochem. J.* 409(1), 77−85.

Benedikt M. Kessler

Henry Wellcome Building for Molecular Physiology, Nuffield Department of Medicine, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK.
Email: bmk@ccmp.ox.ac.uk