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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

*MEROPS name*: trabid peptidase  
*MEROPS classification*: clan CA, family C64, peptidase C64.004  
*Species distribution*: subkingdom Metazoa  
*Reference sequence from*: Homo sapiens (UniProt: Q9UG10)

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

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

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 cytostatin- and metalloc-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.
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, prokaryotic 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

### Table 477.1 Examples* of Ovarian Tumor Domain (OTU) containing proteases with DUB-like activity

| Name(s)     | Origin | UniprotKB | MEROPS | Biological function/Substrates | References |
|-------------|--------|-----------|--------|---------------------------------|------------|
| OTU1 (YOD1, OTUD2, HIN7) | human | Q5VVQ6 | C88    | DUB in ERAD                     | [37]       |
| OTUD1 (DUBA-7) | human | Q5V17 | 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 | 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  |
| VCP1P1 (VCP135) | human | Q96H17 | C64    | DUB in ER, Golgi                | [34,35]    |
| TRABID (ZRANB1) | human | Q9UG10 | 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_CCHF1     | virus  | Q6TQR6 | C87    | de-Ub/ISGylation                 | [47]       |
| OTLD1       | plant  | AT2G27350 | 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.

Accession numbers derived from SwissProt.

Accession number derived from UniGene/NCBI.
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
host-pathogen interactions. It remains to be seen whether any of the vOTUs may also show reactivity to the other interferon-induced Ub1 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 cIAP1, 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-κB (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 (VCIP11) 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 ubiquitous 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].

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