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***Ubiquitin and Ubiquitin-Like Specific Proteases Targeted by Infectious Pathogens:  
Emerging Patterns and Molecular Principles***

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Running Title: Ub/Ubl-specific proteases encoded by pathogens

Key Words: bacteria; hydrolase; ISG15; Nedd8; protease; SUMO; ubiquitin; viruses

Abbreviations: OTU: ovarian tumor domain; UBP: ubiquitin processing protease; UCH:  
ubiquitin C-terminal hydrolase; ULP: ubiquitin-like specific protease; USP: ubiquitin  
specific protease

**Abstract**

Attachment of ubiquitin (Ub) or ubiquitin-like (Ubl) modifiers is a reversible post-translational modification that regulates the fate and function of proteins. In particular, proteolytic enzymes with Ub/Ubl processing activity appear to be more widespread than originally anticipated. It is therefore not surprising that bacterial and viral pathogens have exploited many ways to interfere with Ub/Ubl conjugation, but also de-conjugation. On one hand, pathogens were shown to manipulate host encoded enzymes. On the other hand, pathogen derived sequences of proteases specific for Ub/Ubls are emerging as a common feature shared by many viruses, bacteria and protozoa, and we are at an early stage of understanding how these proteases contribute to the pathogenesis of infection. Whereas some of these proteases share a common origin with mammalian cell encoded hydrolases with specific properties towards Ub/Ubls, most of them have ancient intrinsic functions, such as processing pathogen protein components, and may have acquired the specificity for Ub/Ubls by interacting with mammalian hosts and their immune system throughout evolution. Since many of these proteases are clearly distinct from their mammalian counterparts, they represent attractive targets for drug design against infectious diseases.

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**1. Introduction**

In the course of evolution viral and bacterial pathogens have acquired a series of molecular strategies to promote infection, proliferation and survival during host-pathogen interactions. This aspect has been of considerable interest over many years, since much can be learned about cell biological processes in general by observing how pathogens are capable of manipulating them to their advantage. In addition, a greater understanding of the underlying molecular mechanisms of these processes will continue to provide entry points for drug targets and therapeutic treatment strategies, for which there is a great demand, since many infectious diseases are still not curable.

Pathogens are able to corrupt many of the cell biochemical pathways of the host, one of which is the ubiquitin system that has attracted significant attention in recent years. A reason for this is the fact that to date, over a thousand proteins have been associated with this pathway (reviewed in [1], [2]). Modification by ubiquitin and ubiquitin like proteins is now recognized to be essential for the fate and function of the majority of proteins and can regulate biological processes including protein turnover, receptor internalisation, protein targeting to different cellular compartments, regulation of cell function and cell signalling. There are more than 500 proteins that are known to recognize ubiquitin and attach it to specific substrates (referred to as E2 and E3s), and over 90 deubiquitylating enzymes that can reverse this reaction [3]. This extraordinary diversity reflects the fact that ubiquitin conjugation and deconjugation is a very general regulatory process, which requires to be tightly controlled. Furthermore, an additional complexity of this pathway is based on the existence of many ubiquitin-like molecules (Ubls), such the as small ubiquitin-like modifiers (SUMO1/2/3), neuronal precursor cell expressed, developmentally down-regulated 8 (NEDD8), ubiquitin cross-reactive protein (UCRP, also known as interferon stimulated gene ISG15), ubiquitin-like protein-5 (UBL-5, also known as homologous to ubiquitin-1, Hub1), Ubiquitin D (also known as F-adjacent transcript 10, FAT10/UBD), Fau ubiquitin-like protein (Fau/Fub1), ubiquitin-related modifier 1 (URM1), and Atg8 (GABARAP / GATE16 / MAP-LC3) (reviewed in [4], [1]). Targeting Ub/Ubl conjugation via the E1, E2 and E3 enzyme cascade by viral and bacterial pathogens has been a well recognized theme and the subject of recent reviews [5], [6], [7]. However, removal of ubiquitin or Ubls from substrates by deconjugating enzymes appears to be another biochemical step that viruses and bacteria can interfere with [8], [7]. This is accomplished by proteolytic enzymes, predominantly by cysteine

proteases and a small number of metalloproteases. It has become evident that a vast number of proteases have acquired the ability to process Ub/Ubls, probably far beyond the currently known classifications, as novel enzymes are being discovered as well as new predictions made by recent bioinformatics studies [9], [10], [11]. We shall therefore refer to the classification of proteases into clans and families as proposed by the MEROPS database (<http://merops.sanger.ac.uk/index.htm>) for a more detailed discussion of these enzymes.

In the case of ubiquitin, five classes of enzyme families have been defined to date, including ubiquitin C-terminal hydrolases (UCH – *C12 family*), ubiquitin processing proteases (UBP, also referred to as ubiquitin specific proteases, USP – *C19 family*), Josephin domain containing proteases (JD), ovarian tumor domain containing proteases (OTU – *C64, C85, C87, C88 families*) and metalloproteases (MM) (Figure 1) [12], [3] [13]. The UCH class contains only three members that are small proteins and that were originally identified to process Ub precursors and hydrolyse small chemical groups at the C-terminus of ubiquitin. UBPs (USPs) form the largest enzyme family, and all members share the conserved cysteine and histidine boxes as short motives, but otherwise can be very diverse. Another family of proteases sharing the ovarian tumor domain (OTU) as a common feature is conserved throughout evolution, and is found in ca 80 genes derived from viruses, bacteria, plants and mammals, suggesting their involvement in fundamental biological processes. Deubiquitylating activity was also assigned to a small number of Josephin-domain and JAB metalloprotease domain containing proteases. It is anticipated that many more enzymes may be added to this list, since bioinformatics based prediction algorithms suggest larger families of cysteine proteases including a Permuted Papain fold Peptidases of DsRNA viruses and Eukaryotes (PPPDE) and Wss1p-like metalloproteases (WLM) domains to be potential DUB candidates [10]. Proteolytic enzymes with specificities towards Ubls are classified as ubiquitin-like specific proteases (ULPs) and small ubiquitin-like modifier specific proteases (SENPs), both of which belong to the cysteine protease superfamily (reviewed in [13] [14]). In addition, experimental evidence using activity-based profiling strategies based on Ub/Ubl scaffolds and tandem mass spectrometry retrieved a number of novel gene products (including pathogen derived ones) with Ub/Ubl-specific protease activities, which previously escaped to be predicted by any sequence or structure base alignment algorithms (reviewed in [15],[16],[17]). Such probes mainly detect the ability of proteins to interact with Ub/Ubls with sufficient affinity that suicide inhibition of the catalytic site can occur, thereby providing a list of enzyme candidates. A number of these were subsequently confirmed to exert Ub/Ubl-specific processing activities. Clearly, these findings provide the framework for the biochemical and functional characterisation of novel proteolytic enzymes with Ub/Ubl-specific processing activity.

In this review, we first discuss examples where host cell encoded Ub/Ubl-specific proteases are manipulated by pathogens during the infection process (Table 1). Second, we review pathogen encoded proteases that have Ub/Ubl specific activities and what is known about their function (Table 2), and finally argue that some of them have a common origin with mammalian counterparts, whereas others may have evolved completely independently for different functions within pathogen biogenesis, and may have adapted to host-pathogen interactions throughout evolution.

## 2. Host encoded UB/Ubl specific proteases modulated by pathogens

### *Proteases specific for ubiquitin-like proteins stimulated by interferons*

Infection of most cell types by viruses or bacteria leads to expression of interferon stimulated genes, which include the ubiquitin-like protein ISG15 and one of its specific proteases UBP43 (USP18) (reviewed in [18]). This suggests that they are components of the innate immune system and can contribute to an inherent antimicrobial response. During infection, ISG15 is conjugated to substrate proteins by UBE1L, an ISG15-activating enzyme. This process can be specifically blocked by the influenza B virus encoded protein NS1 [19]. Furthermore, ISG15<sup>-/-</sup> mice have an increased susceptibility to influenza A/B, herpes simplex virus type 1, murine gammaherpesvirus 68 and sindbis virus [20].

Interference with deISG15ylation also affects susceptibility to viral infection as demonstrated in USP18<sup>-/-</sup> mice. Cells derived from these animals did not support replication of a number of viruses, and bacteria infected animals succumbed to an overshooting immune reaction. This was accompanied by increased levels of ISGylation, suggesting a possible involvement of USP18 mediated isopeptidase activity in this process [21]. However, increased susceptibility to infection may be explained through multiple mechanisms, since USP18 was also reported to regulate interferon signaling independently of its ISG15 isopeptidase activity [22]. No example has yet been reported in which USP18 is targeted by pathogens directly, but a number of viral gene products have recently been described to have ISG15 deconjugating activity, therefore dampening the IFN response (discussed below).

In addition, several DUBs encoded by mammalian cells can cross-react with ISG15-specific active site probe, such as USP2, IsoT1 (USP5), IsoT3 (USP13) and USP14, suggesting potential specificity for Ub and ISG15 [23]. However, in the case of USP2 and USP5, no significant ISGylating activity was detected using fluorescence based substrates [24]. The functional relevance of the dual specificity of these enzymes still awaits confirmation with protein based substrates, and has not yet been determined.

A second ubiquitin-like protein, FAT10, is also upregulated upon exposure to IFN or TNF $\alpha$  [25]. Overexpression of FAT10 can induce apoptosis, and was reported to target substrates for proteasomal proteolysis as an alternative pathway to ubiquitin [26]. The IFN dependence of FAT10 expression suggest that this molecule also represents a component of the innate immune defence against pathogens, but its specific role in this process as well as FAT10-specific proteases remain to be examined.

### *USP7 targeted by Herpesviruses*

The ubiquitin specific protease 7 (USP7) was initially described as herpes associated ubiquitin specific protease (HAUSP) based on the initial observation that it interacted with the Epstein–Barr nuclear antigen 1 (EBNA1) and the herpes simplex virus type 1 regulatory protein ICP0 [27]. However, despite that these interactions have been studied in detail [28], their functional significance is not yet completely understood. In the case of EBNA1, the USP7 interaction domain overlaps with the one of the tumour suppressor gene P53, suggesting that EBNA1 may interfere with p53 mediated effects, such as premature apoptosis of infected cells [29]. USP7 also contributes to ICP0 induced

reactivation of gene expression from integrated adeno-associated virus AAV [30]. USP7 is generally involved in processes such as transcriptional regulation, DNA replication, apoptosis, and possible endosomal organisation [31]. It appears that multiple functions of USP7 are exploited by those viruses, and further experimental work is required to shed more light on these mechanisms.

### ***CYLD and bacterial infections***

Cylindromatosis is a benign skin tumour also referred to as turban tumour, and is originated by mutations in the CYLD gene [32]. Biochemical studies revealed that CYLD is a deubiquitylating enzyme acting on TRAF2 and TRAF6, which results in a negative regulation of NF- $\kappa$ B by tumour necrosis factor (TNFR) and toll like receptors (TLRs), suggesting that CYLD plays an important role in controlling inflammation [33], [34], [35]. Recent work uncovered that CYLD<sup>-/-</sup> mice are hypersensitive to infection by Non-typeable *Haemophilus influenza* (NTHi) [36], [37]. CYLD expression is induced in cells infected by this pathogen, and it was suggested to down-regulate NF- $\kappa$ B activation through deubiquitylation of TRAF6 and 7, therefore preventing the formation of an antiviral response of the host. A similar observation was made in an infection model using *Escherichia coli* pneumonia, in which an enhanced innate immune response was observed in CYLD<sup>-/-</sup> mice [38]. An adverse effect was observed when CYLD<sup>-/-</sup> mice were challenged with *Streptococcus pneumoniae* [39]. In this case, CYLD was shown to provoke acute lung injury in the context of infection and therefore contributing to lethality. CYLD was suggested to interfere with the NFAT signalling pathway by deubiquitylating transforming growth factor- $\beta$ -activated kinase 1 (TAK1), and to negatively regulate KK3-p38 kinase-dependent expression of plasminogen activator inhibitor-1 [40] [39]. The example of CYLD having opposing effects in three different infection models in particular highlights the potential challenge that will be faced upon the development of novel therapeutic approaches, which may interfere with one infectious agent, but may promote sensibility to another.

Since there are other Ub/Ubl processing enzymes that are involved in regulating inflammation and the function of immune cells, such as A20 [41], it is expected that additional pathogen derived molecular mechanisms might exist that can interfere with ubiquitin mediated activation of immune responses.

### **3. Ub/Ubl proteases in viral pathogens**

#### ***Coronaviruses (SARS)***

An example of a viral DUB that was discovered through structural bioinformatics is the papain-like protease (PLpro) encoded by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV). Following this prediction, it has been demonstrated that PLpro can indeed cleave polyUb<sub>K48</sub>-chains as well as ISG15 and Nedd8 *in vitro*, but with a preference for ISG15 cleavage [42] [43] [44]. Crystallographic studies confirmed that PLpro shares considerable similarities to the catalytic core domain of the herpesvirus-associated ubiquitin-specific protease (HAUSP) and other already characterized DUBs like USP14 (Figure 2) [45] [43]. It was therefore suggested to belong to the USP family [46]. The role of this enzyme is to process nsp1 to nsp3 from the viral replicase polyprotein [47]. Additionally, PLpro has been shown to block IRF3-dependent antiviral



responses through inhibition of phosphorylation and translocation of IRF3. These two actions were suggested to not be attributable to the protease domain of PLpro, although the results obtained with the catalytic mutants used in this study were inconclusive [48]. Interestingly, these events disturb activation of the type I interferon (IFN) responses, which suggests that PLpro is an important molecule in the infection process. It still remains a challenge to functionally distinguish between the general proteolytic activity and specific isopeptidase activity of PLpro, although more recent protease protection experiments suggest that the putative cytoplasmic domain of nsp3, which includes the PLpro domain, might be exposed and therefore potentially capable of processing ubiquitylated and/or ISGylated host cell substrates [49]. In another human coronavirus, NL63, two papain-like proteases (PLPs), PLP1 and PLP2, have been characterised, which process viral replicase polyproteins essential for RNA synthesis and viral replication [50]. PLP2 shares some similarity with PLpro encoded by SARS-CoV, and has been shown to cleave poly-Ub<sub>K48</sub>-chains *in vitro* [50]. Moreover, PLP2 has deISGylation activity, although it has a preference for Ub over ISG15 [24]. In this case, bioinformatics studies revealed that all coronaviruses encode for at least one papain like protease with deubiquitylating activity [51].

### ***Adenoviruses***

Biochemical experiment on adenovirus-infected cells performed using ubiquitin aldehyde (Ubal) as the bait suggested deubiquitylating properties of Avp (23K proteinase, Ad L3, adenain). During infection, Avp processes viral proteins and appears to deubiquitylate some cellular proteins [52]. In order to perform this task, it requires activation through viral DNA, an 11-amino acid viral peptide and actin as cofactors [53]. Additionally, Avp has been shown to cleave the peptide bond of an ISG15 precursor protein. Despite its structural similarity to Ulp1, a protease that cleaves SUMO, Avp does not have SUMO deconjugating properties [52]. This viral proteinase plays a crucial role in the viral infection process by cleaving virion precursor proteins essential for the infectivity of the virus. In addition, Avp disrupts the cytoskeleton by cleavage of cytokeratin 18 in the cytosol, thereby provoking more efficient cell lysis [53]. Avp is also targeted by nitric oxide (NO), and its inhibition via NO blocks replication of the virus [54]. Nevertheless, the deubiquitylating function of Avp has not been implicated in any physiological processes.

### ***Viruses encoding for ovarian tumour (OTU)-like proteases***

In the family of nairoviruses that includes Crimean-Congo Hemorrhagic Fever (CCHF), Dugbe (DUG) and Nairobi Sheep Disease (NSD) viruses, an OTU-like domain is present within their large (L) protein that also contains the RNA polymerase domain [55], [56]. The L protein of CCHFV has been shown to possess a deubiquitylating and deISGylating activity. It has been demonstrated that this protein directly deconjugates poly-Ub chains and ISG15-conjugated proteins *in vitro*. Moreover, it also decreases levels of ISGylated and ubiquitinated host proteins, when expressed in mammalian cells. All these properties have been observed in L proteins of NSDV and DUGV, when these were expressed in mammalian cells. Moreover, Sindbis viruses over-expressing the OTU-like domain of CCHFV-L showed an increase in infectious pathogenicity in mice as compared to controls. This result has been attributed to neutralisation of the protective effect of ISG15

synthesis in infected cells [55]. Another family of viruses, arteriviruses, including Equine Arteritis (EAV) as well as Porcine Respiratory and Reproductive Syndrome (PRRSV) viruses, encode for an OTU-like domain within the nonstructural protein 2 (nsp2) [55], [56]. Nsp2 cleaves the large viral polyprotein in the nsp2/nsp3 site [57]. When overexpressed in mammalian cells, nsp2 decreases the level of Ub and ISG15 conjugates [55]. In the case of the family of Furoviruses, the OTU-like domain was found in the Rice Stripe Virus (RSV) Zhejiang isolate as well as other RSV isolates, and it is present in the pc1 protein encoded by RNA1 [58]. It has been suggested that a putative role of this domain is the auto-proteolytical cleavage of pc1, which leads to activation of the viral polymerase [58]. Additionally, the deubiquitylating function of pc1 would provide a way to interference with the proteolytic pathway in the host.

### ***Herpesviruses***

Activity-based profiling of herpes simplex virus-1 (HSV-1) infected cells using active site-directed probes with a ubiquitin scaffold combined with tandem mass spectrometry has revealed a virus-encoded USP, UL36<sup>USP</sup>, that corresponds to the N-terminal fragment of the large viral tegument protein UL36 [59]. UL36<sup>USP</sup> cleaves ubiquitin, but not SUMO, ISG15 or Nedd8, and it has specificity towards K<sup>48</sup> linkages of polyubiquitin [59]. The catalytic site of UL36<sup>USP</sup> (Cys65) is conserved throughout the whole family of *Herpesviridae* [59], but there are no homologues of UL36<sup>USP</sup> in eukaryotic cells. Other members of the *Herpesviridae* family such as human cytomegalovirus (CMV), murine cytomegalovirus (MCMV), murine Gammaherpesvirus 68 (MHV), Marek's disease virus (MDV) and pseudorabies virus (PrV) contain UL36<sup>USP</sup> homologues. In most of these viruses it has been shown that the UL36<sup>USP</sup> homologues have deubiquitylating activity *in vitro* [60], [61], [62], [63]. In CMV, the whole 235-kDa high-molecular-weight protein (HMWP) displays a deubiquitylating activity [61], similarly to MHV<sup>USP</sup>, where the polypeptide that is reactive to the ubiquitin active probe is as large as ~250 kDa [64]. However, it is not the case for the HSV-1 UL36<sup>USP</sup>, where the active UL36<sup>USP</sup> is only ~47 kDa. The physiological relevance of these viral DUBs is not yet fully understood, although for two herpesvirus tegument USPs (MDV and PrV), mutation of the catalytic site cysteine affected the rate of viral replication. The expression of MDV<sup>USP</sup> is additionally associated with lymphoma formation and metastasis in chicken [63], whereas mutagenesis of the active site of PrV<sup>USP</sup> inhibits viral neuroinvasion [62], which may be linked to a possible defect in transporting membrane associated virus particles along microtubules [65].

## **4. Ub/Ubl proteases in bacteria and protozoa**

### ***Bacteria***

Although bacteria do not have an intrinsic ubiquitin system, it was found that many bacterial strains contain Ub/Ubl specific proteases. For instance, the *Salmonella* genome encodes for two different proteins with deubiquitylating activities, AvrA and SseL. Both are effector proteins translocated through type three secretion system (TTSS) of *Salmonella* (reviewed in [6]). AvrA deubiquitylates I $\kappa$ B $\alpha$  and  $\beta$ -catenin as demonstrated with GST-tag purified enzyme, thus stabilizing I $\kappa$ B $\alpha$  and  $\beta$ -catenin and inhibiting the NF-

$\kappa$ B inflammatory responses of the host [66]. Similarly, SseL through its deubiquitinating activity suppresses I $\kappa$ B $\alpha$  ubiquitination and thereby prevents NF- $\kappa$ B activation [67]. SseL can deubiquitylate mono- and poly- ubiquitylated substrates and has affinity towards K<sup>63</sup>-poly-Ub chains [68], suggesting that it may play a role in disruption of cellular signaling. In addition, SseL may be involved in delayed cytotoxicity of macrophages that constitutes an important mechanism of *Salmonella* virulence [67].

During infection with *Yersinia*, another enterobacterium that uses a type three secretion system, a virulence factor, referred to as YopJ promotes intracellular survival of the bacterium via inhibition of NF- $\kappa$ B and MAPK, as well as IRF3 signaling pathways [69]. YopJ was first described to act as a deubiquitinase cleaving the K<sup>63</sup>-poly-Ub chains of TRAF2, TRAF3 and TRAF6 [70], [69]. YopJ can also deubiquitylate K<sup>48</sup>-poly-Ub chains of I $\kappa$ B $\alpha$ , thereby preventing its degradation and translocation of NF- $\kappa$ B [71]. It has been proposed that YopJ may also cleave sumoylated proteins [71], but more recent studies [72] also revealed that it also acts as an acetyltransferase to modify serine and threonine residues in the activation loop of MAPKK6, thereby blocking its phosphorylation and activation. In this way, YopJ interferes with the host proinflammatory responses most likely via both, its Ub/Ubl processing properties, but also via its acetylating activity [73], [72]. This finding may represent a common feature that can be found in other proteolytic enzymes, such as the *E. coli* derived TAP protein [74].

Activity-based profiling studies in the *Chlamydia* proteome using ubiquitin based probes identified two active deubiquitylating enzymes of *Chlamydia trachomatis*, ChlaDub1 and ChlaDub2. They have been reported to cleave Ub as well as Nedd8 conjugates [75], but neither their physiological role nor any substrate candidates have yet been discovered. The *Escherichia coli* encoded protease ElaD, has also been identified as a deubiquitylating enzyme specific for ubiquitin, but not for Ubls, like ISG15, Nedd8 or SUMO. ElaD is found in all intestinal strains, but absent from extraintestinal strains of *E.coli*, an observation that may point towards the significance of ElaD in the infection process [76].

### ***Parasitic protozoa***

Although there have been predictions of many putative deubiquitylating enzymes (see review [77]) encoded by medically relevant parasitic Protozoa using bioinformatics studies, only three deubiquitylating enzymes have been reported to date on the basis of their binding to the Ub/Ubl active site probes [78] [79]. All of them have been found via an activity-based Ub/Ubl probe approach and no alternative validation of their DUB activity has been yet carried out. *Plasmodium falciparum* encodes for two deubiquitylating enzymes, PfUCHL3 and PfUCH54, the latter being a homolog of human deubiquitylating enzymes UCH37, UCH-L1 and UCH-L3 (Figure 2). Although the sequence did not reveal a high sequence identity between PfUCH54 and human DUBs, all active site residues have been preserved [78]. Interestingly, PfUCHL3 and PfUCH54 have both deubiquitylating and deNeddylating activity [78] [79], as shown using activity based Ub/Ubl probes. Because PfUCH54 does not have a specific N-terminal sequence that allows it to be secreted, it has been hypothesized that this protein has an important function within the parasite itself, and is not host-targeted [78].

Another protozoa-encoded deubiquitylating enzyme is TgUCHL3 that is expressed by *Toxoplasma gondii*. It is also a homologue of UCH-L3 and was found to be specific to Nedd8 and ubiquitin active site probes, but not SUMO [79]. However, the probes that were used for studying TgUCHL3 and PfUCHL3 reactivity to Nedd8 and SUMO were prepared using Ubl proteins that are more closely related to the human sequences than to their parasitic homologues, therefore the observed reactivities towards the different Ubls should be interpreted with caution. In all these cases, the precise role of these proteases within the context of pathogenicity has not yet been elucidated.

## 5. Common ancestry patterns of UB/Ubl specific proteases involved in host-cell interactions

Although there is no evidence that bacterial organisms do have a ubiquitin system, many pathogens of prokaryotic origin seem to encode for proteins with Ub and Ubl recognition and processing properties. This appears to be the case also for viral pathogens, in which the presence of Ub/Ubl-specific proteases appears to be more widespread than previously thought. Pathogens with larger genomes contain most likely more Ub/Ubl specific proteases, and more advanced bioinformatics approaches are beginning to reveal novel members, as exemplified for the recent discovery of novel bona fide DUBs encoded by EBV [80]. For many of these, we are at an early stage of deciphering their precise functions, but sequence and structure based comparisons with mammalian Ub/Ubl-specific protease families may provide some clues about aspects of their biology. Based on this, pathogen encoded Ub/Ubl proteases would appear to fall into two general categories:

The first one may consist of Ub/Ubl proteases that can be added to the list of already characterised superfamilies (Figure 2). For instance, the family of ubiquitin C-terminal hydrolases (UCHs) contains three mammalian hydrolases, and the more recently characterised proteases encoded by the parasites *Plasmodium falciparum*, *Toxoplasma gondii* and related organisms may form an extended UCH superfamily. A second example may consist of the adenovirus encoded protease adenain, the *Chlamydia* derived ChlaDUB1/2 proteases, *E. coli* derived ElaD, *Salmonella* encoded SseL, *Yersinia* encoded YopJ and similar sequences observed in other pathogens, all of which share some similarities with ULP/SENPs-related proteases that are specific for SUMO and Nedd8 (Figure 2). This superfamily of cysteine proteases has been classified as the CE clan, which targets Ubiquitin and Ubls (MEROPS database [76], but potentially harbours other enzymatic functions as illustrated in the case of YopJ [81] and adenain [52]. A third example is the SARS coronavirus encoded protein PLpro that contains a catalytic core that is structurally related to the ones observed in USP7 and USP14 [45]. A fourth example is the protein family sharing the ovarian tumour domain (OTU), which contains ca 80 members derived from plants, drosophila, bacteria, viruses, yeast and higher eukaryotes ([11], [3]). In all these cases, this may point towards common fundamental ancestry functions that are essential within these organisms. Adaptation mechanisms in the context of host-pathogen interactions may have further diversified their functions, as exemplified in the case of viral OTU containing proteases that have acquired specificity for ISG15, a trait that is not observed for their mammalian counterparts [55].

The second category is represented by novel and unique classes of pathogen encoded Ub/Ubl specific proteases (Figure 2). This is most likely the case for herpes virus encoded tegument derived DUBs (reviewed in [51]). The primary function of these proteases may involve the processing of viral gene products necessary for virus life cycle, and adaptation to Ub specificity as a dual function may have evolved in an evolutionary process to increase the pathogenicity of host-pathogen interactions. Unique structural features of pathogen encoded Ub/Ubl-specific proteases remain attractive entry points for the development of novel antimicrobial drugs, and research efforts in this direction may soon provide novel alternatives to combat a variety of infectious diseases.

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## Figure Legends

**Figure 1: *Scheme of Ub/Ubl-specific proteases.*** Proteases with specificity to Ubiquitin (Ub) or Ubiquitin-like proteins (Ubls) are currently classified into the following protein families: UCHs: Ubiquitin C-terminal hydrolases; USPs: Ubiquitin specific proteases (also referred to as UBPs: Ubiquitin processing proteases); OTUs: ovarian tumour domain containing proteases; MMs: metalloproteases; JDs: Josephin domain containing proteases; SENPs: small ubiquitin-like modifier specific proteases; ULPs: Ubiquitin-like protein specific proteases. See text for a more detailed discussion.

**Figure 2: *Pathogen encoded Ub/Ubl proteases and their relation to mammalian enzymes.*** Although many proteolytic enzymes derived from pathogens such as viruses (red), bacteria and protozoa (green) have unique properties, most of them are related to mammalian Ub/Ubl protease families (white) based on sequence or structure based similarities. Proteins with deubiquitylating enzyme activity encoded by *Herpesviridae* appear to be unique with little or no resemblance to any other Ub/Ubl protease families. See text for a more detailed discussion.

## Table

Table 1. Host encoded Ub/Ub1-specific proteases

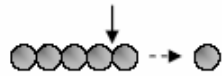

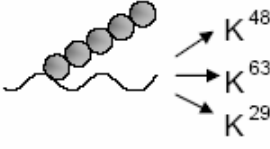




Organism	Pathogen	Protein	Biological function	Host DUB	Biological function of the target protein	Physiological effect	References
Viruses	Herpes simplex virus type 1	ICP0	maintaining balance between the latent and lytic states of a virus	USP7	deubiquitylation	stabilization of ICP0	30, 31
	Epstein-Barr virus	EBNA1	replication, segregation, transcriptional activation of latent virus genomes	USP7	deubiquitylation	stabilization of EBNA1, regulation of its replication function	27, 28, 29
	LCMV, VSV	N.D.	N.D.	USP18	DeISoylation	Regulation of interferon signaling	21,22
Bacteria	Non-Typable <i>Haemophilus influenzae</i>	N.D.	N.D.	CYLD	deubiquitylation	negative regulation for NF- $\kappa$ B dependent inflammation, protection of the host against response to infection	36, 37
	<i>Streptococcus pneumoniae</i>	N.D.	N.D.	CYLD	deubiquitylation	acute lung injury, increased mortality, CYLD deubiquitylates TAK1	39, 40
	<i>Escherichia coli</i>	N.D.	N.D.	CYLD	deubiquitylation	negative regulation for NF- $\kappa$ B dependent inflammation, protection of the host against response to infection	38

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Table

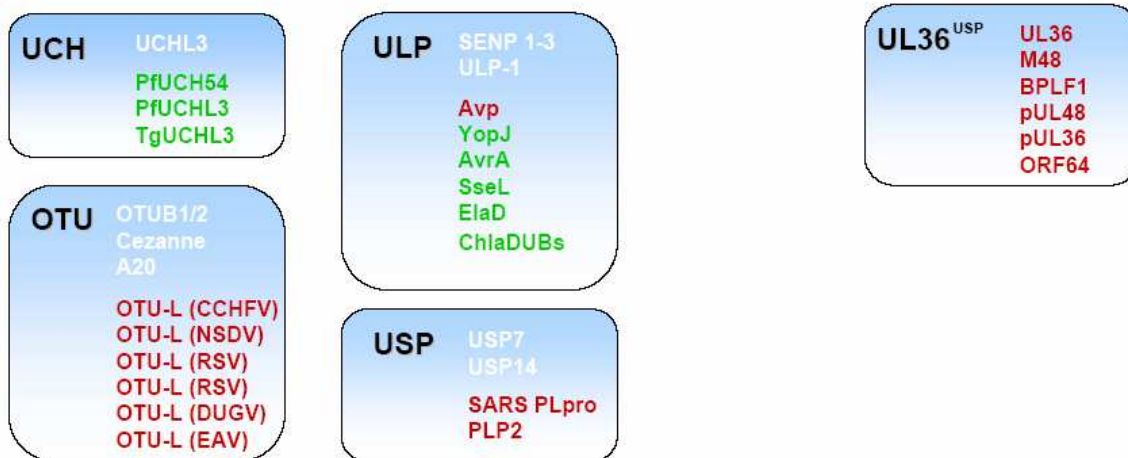
Table 2 - Pathogen encoded Ub/UBI-specific proteases

Organism	Pathogen	Ub/UBI-specific protease	Specificity	Target	Physiological effect	References
Viruses	Coronavirus MERS	PUP2	deubiquitylation, deubiquitylation	N.D.	N.D.	24, 56, 61
	Severe Acute Respiratory Syndrome Coronavirus	SARS-CoV P1pro (P1pro)	deubiquitylation, deubiquitylation, deubiquitylation	N.D.	N.D.	42, 43, 44, 45, 46, 47, 48
	Adenovirus	Adenovir (Arg. L3 22K protease)	deubiquitylation, deubiquitylation	N.D.	N.D.	52, 53, 54
	Chinese Congo hemorrhagic fever virus	OTU L (COHFV-L)	deubiquitylation, deubiquitylation	N.D.	N.D.	55
	Human Sheep Disease Virus	OTU L (NSFV-L)	deubiquitylation, deubiquitylation	N.D.	N.D.	55
	Doghe virus	OTU L (DGHV-L)	deubiquitylation, deubiquitylation	N.D.	N.D.	55
	Porcine respiratory and reproductive syndrome virus	OTU L (PRRSV-L, Nsp2)	deubiquitylation, deubiquitylation	N.D.	N.D.	55, 56
	Equine arteritis virus	OTU L (EAV-L, Nsp2)	deubiquitylation, deubiquitylation	N.D.	N.D.	55, 56
	Non-retrovirus Zhejiang	OTU L (NSV-L, p1)	predicted deubiquitylation	N.D.	N.D.	58
	Hepes delta virus 1	UL38	deubiquitylation	N.D.	N.D.	59
	Marine cytomegalovirus	M88	deubiquitylation	N.D.	N.D.	60
	Epidemiological virus	BPU1, BSLP1, BSLP1	deubiquitylation	N.D.	N.D.	60, 60
	Human cytomegalovirus	pUL89	deubiquitylation	N.D.	N.D.	61
	Marine Gammaherpesvirus 88	ORF64	deubiquitylation	N.D.	N.D.	64
	Pseudotuberculosis virus	pUL20	predicted deubiquitylation	N.D.	regulated in viral replication and reactivation	62
	Marek's disease virus	gB <sup>MDV</sup>	deubiquitylation	N.D.	viral reactivation	63
Bacteria	<i>Salmonella</i> sp.	AvhA	deubiquitylation	I $\kappa$ B $\alpha$ , p-catenin	inhibition of the host inflammatory responses	66
	<i>Salmonella</i> sp.	SseK	deubiquitylation	N.D.	delayed cytotoxicity of macrophages	67, 68
	<i>Yersinia</i> sp.	YopJ	acylation, deubiquitylation, deubiquitylation	TRAF3, TRAF2, TRAF6 and I $\kappa$ B $\alpha$	inhibition of the host inflammatory responses	69, 70, 71, 72, 73
	<i>Chlamydia trachomatis</i>	CtHsc70 and CtHsc70-2	deubiquitylation, deubiquitylation	N.D.	N.D.	75
	<i>Escherichia coli</i>	Shd	deubiquitylation	N.D.	N.D.	76
Protozoa	<i>Plasmodium falciparum</i>	PTCH54	deubiquitylation, deubiquitylation	N.D.	N.D.	78
	<i>Plasmodium falciparum</i>	PTCH23	deubiquitylation, deubiquitylation	N.D.	N.D.	76, 79
	<i>Toxoplasma gondii</i>	TgUCH3	deubiquitylation, deubiquitylation	N.D.	N.D.	79

Type of Ub/Ubl trees	Classification	Protease family*
Ub 	UCH	C12
Ub 	USP / OTU / MM / JD	USP: C19 OTU: C64, C85, C87, C88 MM: M67 JD: C86
Ub 		
SUMO 		
SUMO 	SEN1-3 (ULP)	C48
Nedd8 	ULP	C48
ISG15 		

\*MEROPS database  
(<http://merops.sanger.ac.uk/index.htm>)

A

*Related to mammalian Ub/Ubl protease families**Unrelated**Legend*

Mammalian DUBs  
 Viral DUBs  
 Bacterial and Protozoa DUBs  
 DUB categories

ACCEPTED