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Review

Structural diversity of the CE-clan proteases in bacteria to disarm host ubiquitin defenses

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Ubiquitin (Ub) and ubiquitin-like (UbL) modifications are critical regulators of multiple cellular processes in eukaryotes. These modifications are dynamically controlled by proteases that balance conjugation and deconjugation. In eukaryotes, these proteases include deubiquitinases (DUBs), mostly belonging to the CA-clan of cysteine proteases, and ubiquitin-like proteases (ULPs), belonging to the CE-clan proteases. Intriguingly, infectious bacteria exploit the CE-clan protease fold to generate deubiquitinating activities to disarm the immune system and degradation defenses of the host during infection. In this review, we explore the substrate preferences encoded within the CE-clan proteases and the structural determinants in the protease fold behind its selectivity, in particular those from infectious bacteria and viruses. Understanding this protease family provides crucial insights into the molecular mechanisms underlying infection and transmission of pathogenic organisms.

Highlights

Ubiquitin (Ub) and ubiquitin-like (UbL) modifiers are essential in eukaryotes. This is partly controlled by deubiquitinases (DUBs) and ubiquitin-like proteases (ULPs), the latter belonging to the CE-clan of cysteine proteases, balancing the conjugation and deconjugation of cellular targets.

In eukaryotes, CE-clan proteases remove small ubiquitin-like modifier (SUMO) and neural precursor cell-expressed developmentally downregulated gene 8 (Nedd8) from protein targets. However, bacterial and viral CE-clan proteases have acquired deubiquitinating activities, emerging as pivotal players in host–pathogen interactions by undermining and evading the host immune system.

The collaborative action of the variable regions within the CE protease structure determines their substrate selectivity and enables them to target various Ub or UbL moieties.

The development of strategies to disrupt the ability of the protease to manipulate host Ub and UbL pathways could lead to promising novel treatments against infections.

Families of deconjugating enzymes across species

Ub and UbL proteins (see Glossary), such as **small ubiquitin-related modifier (SUMO)** or **neural precursor cell-expressed developmentally down-regulated gene 8 (Nedd8)**, function as post-translational modifications (PTMs) and participate in a plethora of cellular pathways in eukaryotes, including protein homeostasis and trafficking, chromatin regulation, DNA repair, proteasomal degradation, and more [1–4]. The balance between conjugation and deconjugation of these PTMs is vital to maintain proper cell functioning [5]. E3 ligases are responsible for their conjugation to target proteins, whereas proteases mediate the deconjugation steps. The tight interplay between these two processes regulates the intricate network of interactions defining what is called the 'Ub and UbL code' [6].

Briefly, the eukaryotic conjugation/deconjugation cycle begins with **DUBs** and **ULPs** activating Ub and UbL precursors, respectively. After activation, a cascade of enzymes (E1, E2, and E3) work together to conjugate the matured Ub or UbL to the target protein, forming an isopeptide bond. Finally, DUBs and ULPs are responsible for deconjugating Ub or UbL by cleaving this bond. These proteases have a dual role in maintaining the free pool of Ub/UbLs inside the cell: they process newly synthesized precursors and recycle Ub/UbLs from modified proteins. While eukaryotic DUBs show a broad range of activities, including deubiquitination, deSUMOylation, deNEDDylation, delSGylation, or deUFMylation, ULPs exclusively participate in deSUMOylation and deNEDDylation. Detailed reviews of the Ub/UbL conjugation pathway can be found elsewhere [4,7].

There are eight families of DUBs, each distinguished by a unique structural fold and exhibiting different cleavage preferences [8–10]. Noteworthy, some DUBs can display crossreactivity with other types of UbL, as explored below. Despite such structural and functional differences, seven families are cysteine proteases, belonging to the CA-clan of proteases, with one outlier

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being a zinc-dependent metalloproteinase [11]. Detailed reviews of DUB activities are available elsewhere [12,13].

Among UbL modifications, SUMO is the most widely used and best characterized [14]. However, the number of SUMO proteases is limited compared with the number of DUBs involved in Ub regulation. Enzymes responsible for SUMO processing and deconjugation are called ULPs in yeast, or Sentrin-specific proteases (SENPs) in mammals. They belong to the **CE-clan of cysteine proteases** and share a common papain-like fold. The first described SUMO protease was UbL-specific protease 1 (Ulp1) from *Saccharomyces cerevisiae* [15,16]. Subsequently, a yeast homolog, Ulp2, and mammalian orthologs, the SENPs, were identified through sequence similarity searches [17,18]. The human SENP family comprises six SUMO proteases, SENP1–3 and SENP5–7, and one deNEDDylase, SENP8/NEDP1 (Box 1). Resembling DUBs, SENPs and ULPs have specific substrate selectivity in eukaryotes.

Although most prokaryotes lack Ub or UbL modification pathways, a growing number of bacterial UbL conjugation/deconjugation systems have been identified and characterized in recent years [19,20]. Nevertheless, the presence of these proteases in bacteria is particularly intriguing given the ability of pathogenic and symbiotic bacteria, as well as viruses, to evolve mechanisms that manipulate the Ub and UbL code of the host, thereby enhancing their survival and persistence [21]. While most bacterial DUBs belong to the CA-clan proteases [22,23], the CE-clan proteases are also present in both viruses and bacteria (Figure 1A) [24]. Interestingly, CE-clan proteases in bacteria have evolved broader substrate specificity compared with their eukaryotic counterparts, enabling them to recognize not only UbLs, but also Ub chains. Some proteases have even evolved entirely new activities, such as acetyltransferase functions [21,24–26]. This contrasts with the lack of CE-clan DUBs in eukaryotes. Therefore, the CE-clan proteases exemplify a notable case of protein evolution; despite being a small and conserved fold, these proteases have diversified to target different substrates while maintaining high specificity within each member. Unveiling the mechanisms behind this remarkable adaptation remains an active area of research.

In this review, we explore the structural versatility of CE-clan proteases and their preference for Ub or UbLs, with a particular emphasis on their role in pathogenic and symbiotic bacteria and viruses.

Box 1. Human SENP family

The SENP family is divided into four groups based on sequence similarity: SENP1-SENP2, SENP3-SENP5, SENP6-SENP7, and SENP8/NEDP1 [87]. SENPs can also be clustered regarding their relationship with their yeast homologs, with SENP1, SENP2, SENP3, and SENP5 belonging to the Ulp1 branch and SENP6 and SENP7 to the Ulp2 branch. SENP8/NEDP1 is the only member of the SENP family that lacks deSUMOylation activity, with its substrate being Nedd8 instead. While all SENPs share a common catalytic domain, each has a specialized function, with different substrate selectivity, subcellular localization, and regulatory pathways [88]. Often, N-terminal extensions have an important role in regulation and localization through the establishment of protein–protein interactions, while substrate specificity is intrinsically encoded in the protease domain. Alternatively, SENP activities and localization can be modulated by alternative splicing and PTMs, further expanding the regulatory repertoire of SENP proteins [89–92].

Despite these differences, SENPs are generally intimately associated with the nucleus, because SUMO has a key role in ribosome maturation, DNA repair, and gene expression [93–95]. SENP1 and SENP2 primarily localize in the nucleus, and disruptions in their N-terminal domains can lead to mislocalization and altered SUMO conjugate patterns. SENP3 and SENP5 are preferentially targeted to the nucleolus, where they demonstrate fundamental activities for 60S (SENP3) and 40S (SENP3/5) ribosome maturation pathways [96]. SENP6 and SENP7 are predominantly found in the nucleoplasm, with at least partial localization to chromatin, underscoring their involvement in nuclear functions [97,98]. Notably, the catalytic domains of SENP6 and SENP7 have four conserved loop insertions that are missing in other SENPs [82]. One such loops (Loop 1) has been described to account for their preference for cleaving SUMO2/3-conjugated and di- and poly-SUMO2 [81,83]. Finally, SENP8/NEDP1 occupies a distinct niche within the realm of SENP proteases. Despite its classification within the broader CE-clan protease group, SENP8/NEDP1 stands out as a deNEDDylase because it exhibits specificity toward UbL Nedd8 and it is not active against SUMO. Unlike some of its counterparts, SENP8 is less characterized, with its precise roles in cellular processes still being elucidated [99,100].

Glossary

CE-clan proteases: unique group of proteases categorized by their structure and function. They belong to the large family of papain-like cysteine proteases, which use a catalytic triad formed by a cysteine, histidine, and aspartic acid or asparagine. In eukaryotes, CE-clan proteases specifically target UbL modifiers, such as SUMO or Nedd8; however, in infectious bacteria and viruses, CE-clan proteases have evolved to cleave Ub or have even acquired different activities, such as acetyl transferase.

Deubiquitinating proteases (DUBs): cellular enzymes that remove the 'ubiquitin tag' from proteins, which often signals a protein for breakdown. DUBs protect proteins from degradation. Their precise actions help regulate protein levels and influence many vital processes in the cell. Seven different families of DUBs have been discovered in eukaryotes.

Neural precursor cell-expressed developmentally downregulated gene 8 (Nedd8): ubiquitin-like modifier closely related to Ub (58% identity), but unlike Ub, which often targets proteins for degradation, Nedd8 has diverse effects on its target proteins. Neddylation can change the function, stability, or location of a protein within the cell, influencing many cellular processes.

Small ubiquitin-like modifier (SUMO): UbL modifier related to Ub (~18% identity) that modifies the function, stability, or location of the protein within the cell. SUMOylation has a key role in many cellular activities, including DNA repair and gene expression.

Ubiquitin (Ub) and ubiquitin-like (UbL) modifiers: Ub is a post-translational modifier that can be covalently attached to protein substrates in eukaryotes. Polyubiquitin chains can regulate the half-life of a protein by targeting it for destruction by the 26S proteasome (the major recycling machinery in the cell). UbL modifiers form a family of proteins, similar to Ub, with various cellular effects, including stabilization and changes in function. Both Ub and UbL modifiers are essential for regulating many cellular processes.

Ubiquitin-like proteases (ULP and SENP family): in eukaryotes, CE-clan proteases are commonly known as the ULP family and the SENP family in mammals. They cleave the isopeptidic bond between SUMO and its protein target, reversing the effects of

The ability of these proteases to evolve and adapt underscores the outstanding flexibility of bacterial and viral survival mechanisms, while providing a remarkable example of protein evolution. This ability also highlights the substrate recognition mechanisms that these proteases use,

SUMOylation, thereby influencing cellular processes and determining the fate of protein substrates.

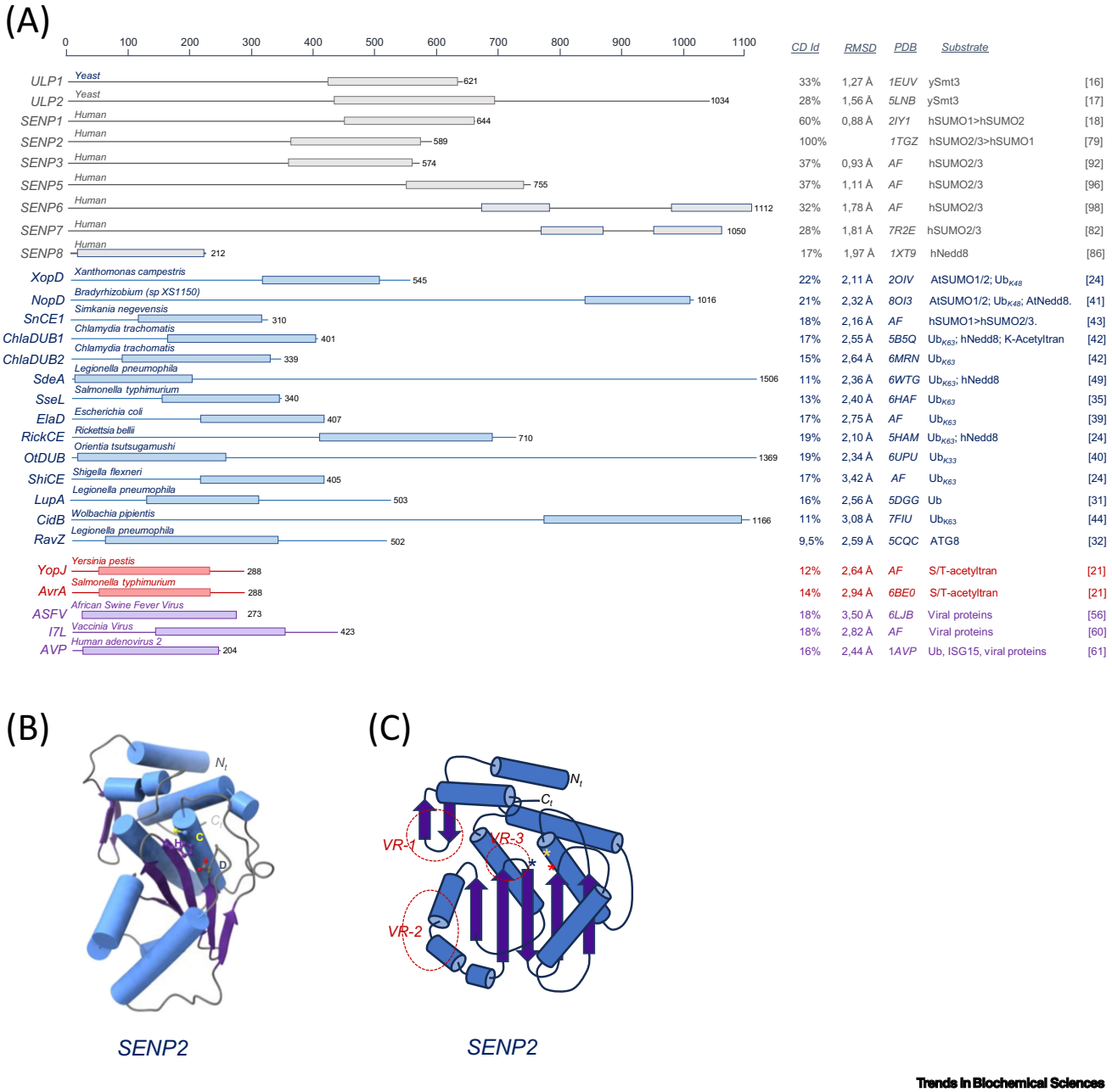

Figure 1. Structural diversity of the CE-clan proteases. (A) CE-clan protease members. Schematic of examples of CE-clan protease members, indicating the length and approximate position of the catalytic domain (rectangle) within the full-length sequence. The name of the CE-clan protease and the organism is indicated. CD id indicates the sequence identity of the catalytic domain in terms of human SENP2. RMSD indicates the structural root-mean square deviation compared with human SENP2. The PDB code is also indicated, unless the structure was based on an Alphafold-2 model (AF). Substrate preference and references are also indicated. (B) Cartoon representation of the human SENP2 structure. Helices and strands are represented by tube cylinders or arrows, respectively. The catalytic triad is labeled and shown in stick representation. (C) Topology cartoon representation of the SENP2 structure depicting the connectivity of the secondary structure elements of a CE-clan protease. The three variable regions are displayed, as well as the catalytic residues location (colored asterisks). See [16–18,21,24,31,32,35,39–44,49,56,60,61,79,82,86,92,96,98].

which are central to their function in host–pathogen interactions. The study of bacterial and viral Ub/UbL proteases has significantly increased our understanding of the infection process, revealing these enzymes as promising targets for developing infection-fighting strategies. Consequently, a deeper understanding of their structural intricacies may offer valuable insights into the molecular mechanisms driving infection and transmission.

Bacterial and viral CE-clan proteases

In some instances, pathogenic bacteria and viruses rely on effector factors to manipulate host Ub pathways, thus facilitating infection and persistence [21,27]. Effector proteins often disrupt the eukaryotic Ub and UbL systems by targeting components such as E3 Ub ligases or DUBs. This behavior is exemplified by CE-clan proteases in infectious bacteria [22,24]. Despite their eukaryotic counterparts being considered ULPs, these bacterial effectors manipulate the host Ub system and, therefore, should be functionally classified as DUBs.

The origin of CE-clan proteases in bacteria and viruses is a topic of ongoing research. It remains unclear whether these proteases were inherited from a common ancestor or acquired through horizontal gene transfer from the host for adaptative advantages [28–30]. Evolutionary pressures likely favored broad substrate specificity to enhance the versatility of these proteases in hijacking host Ub and UbL systems. This adaptability allows pathogens to efficiently manipulate various host cellular processes with a limited set of enzymes, providing a significant advantage in diverse environments [21,27]. The involvement of proteases in cellular regulation, stress responses, and host–pathogen interactions highlight the intricate interplay between their enzymatic activity and various biological processes.

Bacterial CE-clan proteases

Although bacteria lack Ub, SUMO, or Nedd8, they have CE-clan proteases exhibiting a wide range of enzymatic activities (Figure 1A). These proteases are found in pathogenic bacteria and in bacteria that reside inside eukaryotic cells (i.e., plant symbionts), and have pivotal roles in manipulating the eukaryotic host Ub- and UbL-dependent pathways [21]. Examples of these CE family effectors include LegCE, LupA, LegA15/AnkH, and RavZ from *Legionella pneumophila* [24,31–34], SseL of *Salmonella typhimurium* [35,36], XopD of *Xanthomonas campestris* [24,37,38], ElaD of *Escherichia coli* [39], RickCE from *Ricksettia bellii* [24], OtDUB from *Orienta tsutsugamushi* [40], ShiCE from *Shigella flexneri* [24], NopD from *Bradyrhizobium* [41], ChlaDUB1 from *Chlamydia trachomatis* [42], SnCE1 from *Simkania negevensis* [43], CidB from *Wolbachia pipientis* [44,45], and EmcB from *Coxiella burnetii* [46]. Thus, the high diversity of bacterial CE-clan proteases adds an intriguing evolutionary perspective and they represent a diverse group of enzymes with multifaceted roles in host–pathogen interactions.

Similar to their eukaryotic counterparts, bacterial CE-clan proteases show tight substrate selectivity. However, and despite the high structural conservation and similarity with the human SENP/ULP family, they have evolved specificity against other substrates beyond SUMO and, indeed, most bacterial CE-clan proteases are specific for Ub instead. For instance, effectors, such as ChlaDUB1 from *C. trachomatis*, have both DUB and acetyltransferase activities, allowing them to hijack host signaling pathways and evade immune responses [24,42,47,48]. A structural comparison between bacterial protease effectors from different organisms also confirms a high versatility of the CE-clan catalytic domain to acquire the capability to cleavage polyubiquitin chain linkages [21]. *S. Typhimurium* SseL, *E. coli* ElaD, *Wolbachia* CidB, and *Coxiella* EmcB are other examples that exhibit specificity toward Ub, targeting specific linkages, such as K63-linked polyUb chains, to modulate host immune signaling [35,39,45,46].

Noteworthy, some bacterial CE-clan proteases have the unique trait of encoding broad specificity toward Ub and Nedd8, such as SidE family effectors from *L. pneumophila* [49], RickCE from *Rickettsia*, or ChlaDUB1 from *C. trachomatis* [24,42]; toward Ub and SUMO, such as XopD from *X. campestris* [24], or SnCE1 from *Simkania* [43]; and even toward Ub, SUMO and Nedd8, such as NopD from *Bradyrhizobium* [41], indicating a high evolutionary pressure acting on these proteins. EmcB from *C. burnetii* also shows weak dual activity against Nedd8, but remains poorly characterized [46]. These unique deconjugation activities highlight the plasticity of the optimized protein fold underscoring its significance in bacterial virulence. The structural basis of this plasticity is further analyzed later.

Viral CE-clan proteases

Viruses also encode DUBs that manipulate the host Ub system to counteract the biological actions mediated by E3 ligases, such as proteasomal degradation or immune response [50–53]. These viral DUBs have crucial roles in virus life cycles and defense mechanisms, and, in most cases, are vital for processing polyproteins from the virus into mature forms. Similar to bacterial DUBs, most viral proteases belong to the CA-clan, although several examples of CE-clan viral proteases have been described.

Examples of CE-clan proteases from viruses include the protease from African swine fever virus (ASFV) [54–57], the I7L protease from mpox virus [58–60], and the human adenovirus proteinase (AVP) [61–64] (Figure 1A). ASFV affects domestic and wild boar, resulting in deadly infections; its protease is implicated in the processing of some core shell virion proteins [54–56,65]. The I7L protease from mpox virus also has a pivotal role in virus replication by maturating virions and processing core proteins. Although its structure remains unsolved, modeling efforts have been used to identify potential inhibitors against infections [58]. The AVP viral protease is also involved in processing precursor viral proteins and shows activity against both Ub and ISG15, but does not cleave SUMO substrates [10,61].

Role of CE-clan proteases in infection

As mentioned earlier, DUBs and ULPs are key players in host–pathogen interactions by enabling pathogens to exploit Ub/UbL host pathways to progress infection and evade the immune system [21,22]. The heavy reliance of the host immune response on the Ub modification system makes it an ideal target for bacterial effectors [27]. This strategy is emerging as a common mechanism of viral and bacterial virulence, enhancing their survival and persistence. Additionally, these proteases also have crucial roles in viral protein cleavage, making them essential for viral replication [66–68]. Considering the vital role of these enzymes during infection and persistence, the generation and characterization of inhibitors suggest a promising therapeutic strategy for combating and preventing infectious diseases [58,69,70].

These proteases can interfere with a variety of mechanisms inside host cells, including hijacking trafficking pathways, suppressing immune responses, and shielding against degradation signals (Figure 2) [21,22,71]. For instance, *C. trachomatis* depends entirely on the host for its survival, suggesting that this pathogen uses DUBs to regulate the function and expression of both host and bacterial proteins [42]. Specifically, ChlaDUB1 and ChlaDUB2 proteases from *C. trachomatis* disrupt cell trafficking, leading to Golgi apparatus fragmentation through the targeting of K63-linked Ub chains [48]. The effector EmcB from *C. burnetii* degrades K63 Ub-linked chains to sabotage immune detection, thereby suppressing type I interferon production and enabling evasion of the immune system and persistence within host cells [46]. Similarly, SseL from *Salmonella* dampens the immune response by reducing NF-κB signaling [35,72]. Another example is the protease RavZ from *Legionella*, which prevents the formation of the

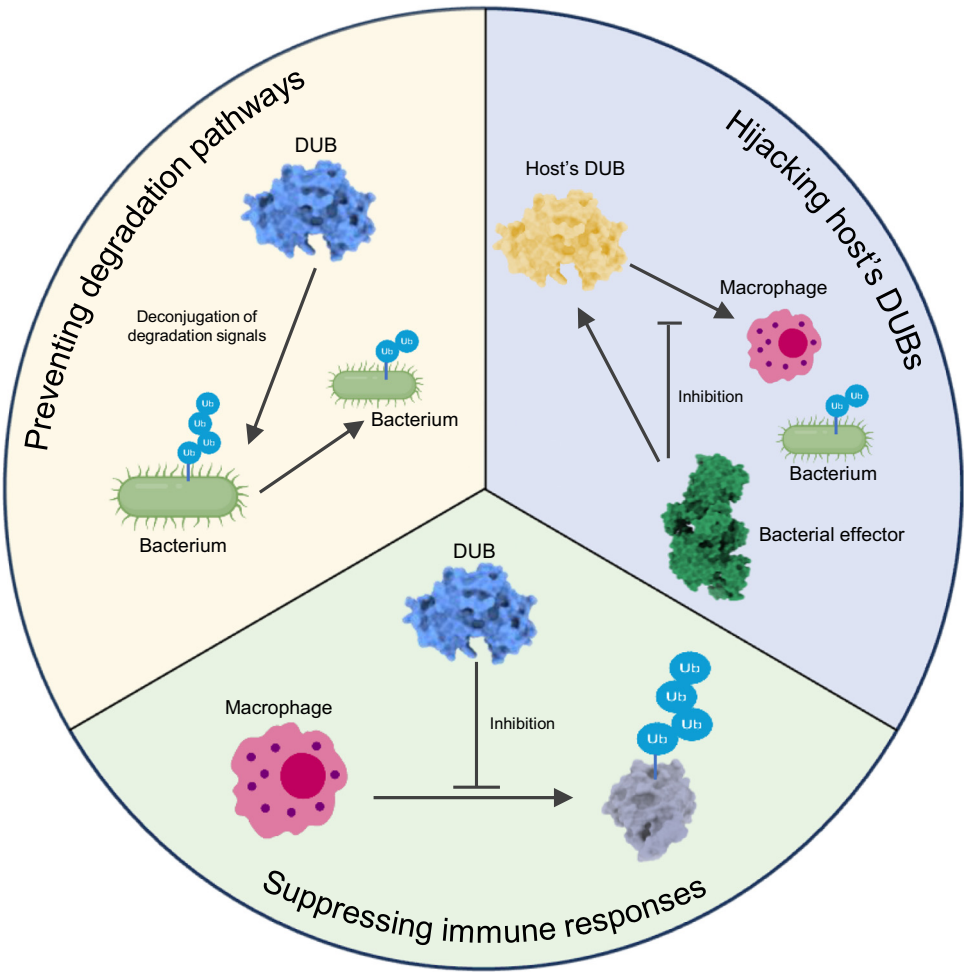

Figure 2. Mechanisms of deubiquitinases (DUBs) during bacterial and viral infections. A schematic illustrating the various strategies used by bacterial and viral effectors during infection. These mechanisms include preventing degradation pathways, suppressing immune responses and hijacking the DUBs of the host.

autophagosome by direct modification of the UbL modifier Atg8/LC3 [32]. Other studies have suggested that this protease is involved in clearing Ub from bacteria-containing vacuoles, although its direct DUB activity has yet to be demonstrated [34,73]. A viral example is ASFV protease from ASFV, which helps to overcome the host immune system by interfering with type I Interferon, thus facilitating viral transmission and replication [53,65,69]. Many of these proteases also have critical roles in various infection and transmission stages, including cleavage of viral proteins essential for replication [51].

The I7L protease from mpox virus serves as an illustrative example of a promising antiviral target for drug development. Obtaining detailed structural information on these proteases is vital for the generation and design of such compounds. Recent studies used structural models of this protease to screen and identify potential inhibitors of the I7L protease [58,70]. While the efficacy of those candidates must be experimentally validated, these initial findings provide a valuable foundation for the development of new antiviral therapies. Advancing this research could lead to effective treatments against mpox and potentially other related viral or bacterial infections [54,58,69,70].

Thus, the central role of bacterial and viral CE-clan proteases in host–pathogen interactions may not only open new avenues to combat infections, but also lead to the development of therapeutic strategies for human diseases associated with dysregulated protein modification pathways [53].

Structural characterization of the CE protease fold

CE-clan proteases share a common cysteine protease papain-like fold featuring a central mixed β-sheet sandwiched by helical elements on both sides (Figure 1B). The protease active site is formed by a catalytic triad of His, Asp (or Gln), and Cys residues [29,74]. As in other papain-like proteases, these residues are located in a cleft between two structural subdomains: a β-subdomain that contains His and Asp (or Gln) residues, and an α-subdomain comprising a helical bundle that carries the catalytic Cys. Interestingly, this structural fold is similar to that of the CA-clan [28], the major family group of DUBs, but with the order of the subdomains inverted.

Three variable regions (VRs), originally termed VR-1, VR-2, and VR-3 [24], define the substrate recognition site near the catalytic cleft. Sequence variations within these regions are sufficient to modify substrate specificity. Due to their solvent exposure, the VRs can tolerate sequence variations, extensions, and deletions without affecting the core protease structure. This inherent plasticity is evident in the 3D structure of the VRs (Figure 3).

Additionally, unstructured segments upstream or downstream of the catalytic domain may contribute to substrate recognition. In some cases, these segments have been reported to contain SUMO-interacting motifs (SIMs), potentially increasing enzyme affinity for SUMOylated substrates such as SENP6 or ULP2 [75,76]. However, these segments likely have a more significant role in localization and protease targeting to specific subcellular locations, rather than in direct substrate docking.

Variable regions inside the CE protease fold

The first variable region (VR-1) of CE proteases is located near the N terminus of the catalytic domain (Figure 3). In the human SENP family, this region forms a β-hairpin that interacts with the SUMO loop region located after the fourth strand of the β-sheet via electrostatic interactions [77–80]. Remarkably, in human SENP6 and SENP7, there is a short eight-residue insertion in the VR-1, called Loop1, which is essential for activity and acts as a determinant of SUMO isoform specificity [81–84] (Box 2). In bacterial relatives, VR-1 exhibits higher structural diversity. For instance, VR-1 is intrinsically disordered in NopD, SseL, and ElaD (according to AlphaFold models) and absent in ChlaDUB1 [24,35,39,41,43,85]. In XopD, a long unstructured tail acts as VR-1, having a key role in its dual substrate recognition for SUMO and Ub [24]. By contrast, VR-1 is absent in SdeA, which also displays dual activity [24,25,49]. This diversity aligns with the observed shift from deSUMOylation to deubiquitination activities in bacterial CE-clan proteases.

VR-2 is formed by a helical-rich extension between the first and second strand of the β-sheet core of the catalytic domain (Figure 3). This region can vary from a few residues, around 30 in SENP1 [77,78], up to 100 in the bacterial RickCE [24]. VR-2 is usually the major contributor (in terms of buried surface) to substrate docking through hydrophobic contacts with the β-sheet of UbL moieties. In the human and yeast SENP/ULP family, VR-2 comprises only two α-helices of variable lengths [15,78,79,82,83]. Once again, bacterial proteases exhibit higher structural diversity in this region, with RickCE forming a large helical domain [24]. Interestingly, NopD and XopD resemble their human and yeast counterparts [24,38,41], while VR-2 of SdeA forms a small two-stranded β-sheet [24,25,49] (Figure 3). Importantly, the helical tendency of this region suggests structural complementarity with the Ub/UbL fold.

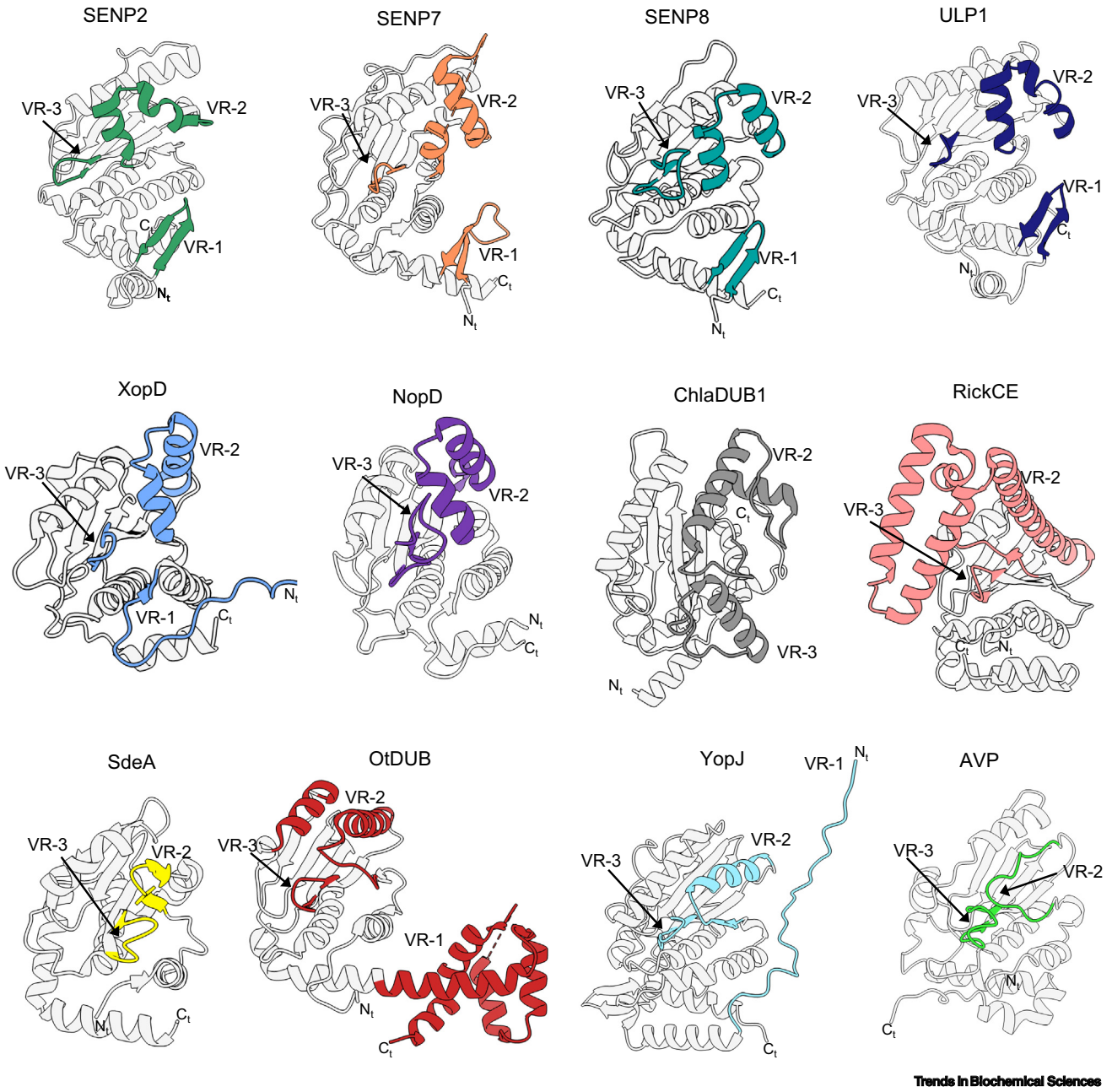

Figure 3. Structural diversity of the Variable Regions (VR)s) of some CE-clan proteases. Cartoon of SENP2 (PDB: 1TH0), SENP7 (PDB: 7R2E), SENP8/Den1 (PDB: 1XT9), ULP1 (PDB: 1DTD), XopD (PDB: 5JP3), NopD (PDB: 8OI3), ChlaDUB1 (PDB: 6GZS), RickCE (AlphaFold), SdeA (PDB: 6WTG), OtDUB (PDB: 6UPU), YopJ (AlphaFold), and AVP (PDB: 1AVP). The three VRs (VR-1, VR-2, and VR-3) are indicated and are illustrated in various colors. The N and C termini are also indicated.

VR-3 is located in a loop connecting the second and third antiparallel β-strands of the central β-sheet, situated next to the catalytic site (Figure 3). In human and yeast SENP/ULPs, VR-3 typically forms a short hairpin loop, except in SENP8/NEDP1, which has a longer loop with seven additional residues important for guiding the Nedd8 C-terminal tail [86]. A higher VR-3 diversity is also observed in bacterial CE proteases, with configurations ranging from small loops in RickCE, SseL, OtDUB, and SdeA, which form minimal contacts with the UbL C-terminal tail, to a longer

Box 2. Discrimination between SUMO1 and SUMO2/3 isoforms in human SENPs

The SENP family constitutes another illustrative example of how minimal changes in the sequence and structure of the protease fold can encode distinct deSUMOylation activities. For instance, it is well known that SENP6/7 prefer to deconjugate SUMO2/3 from substrates, while SENP1 is more involved in maturating SUMO1 and SUMO2 [77,83,97]. Subtle hydrophobic and hydrostatic interactions help to accommodate the SUMO moiety near the active site and, together with small changes in protein sequence, they discriminate between isoforms and paralogues.

Even though SENP1 and SENP2 maintain a 41% sequence similarity, they have different preferences in terms of substrate processing. While SENP1 prefers to process SUMO1 versus SUMO2/3, SENP2 processes SUMO2 with higher capacity [77,79,80,97]. By contrast, both SENP1 and SENP2 can deconjugate SUMO1 and SUMO2/3 from substrates with similar efficiency, indicating that the burden of recognition mostly relies in the C-terminal tail of SUMO. Recently, it was shown that phosphorylation of Y270 of SENP1 can affect the interaction between SUMO isoforms [89], emphasizing the role of the unstructured N-terminal region of SENPs in isoform specificity.

In the case of SENP6 and SENP7, the Ulp2 branch SUMO proteases, there is a strong preference for deconjugation of SUMO2/3 substrates and poly-SUMO chains [81–83]. Structural and biochemical analysis determined that Loop1 in the VR-1 is a determinant for discriminating between SUMO1 and SUMO2/3 isoforms as it moves toward the SUMO2 surface, interacting with a negative patch present in SUMO2 but not in SUMO1 [84]. Deletion of this loop results in diminished activity in deconjugation reactions, highlighting its importance for substrate specificity within the CE-clan proteases. Moreover, the presence of SIM domains in the N-terminal region have also been reported, which may support the recruitment of poly-SUMOylated substrates [75,76].

In summary, the specificity of the different members of the human SENP deSUMOylating family toward distinct SUMO isoforms can be traced back to specific regions within the interface with the SUMO globular domain. These regions may be further complemented by motifs found in the N-terminal regions, which aid localization of the proteases to specific cellular compartments [89]. In our opinion, understanding these molecular mechanisms is the first step toward the development of inhibitors and therapeutic strategies targeting deSUMOylation pathways.

helical region, such in ChlaDUB1 [24,25,35,40,49,85]. Interestingly, in rhizobial NopD, the VR-3 loop has a critical role in guiding the C-terminal tail of AtSUMO2, Ub, or Nedd8 into the catalytic active site, resembling the role of VR-3 in human SENP8/NEDP1 [86].

These three regions collaborate to confer substrate selectivity for a wide range of UbL modifiers within the CE-clan protease family. Consequently, different orientations of Ub are observed on the bacterial CE-clan protease surface, depending on the interfaces involved in the interaction (Figure 4). For example, SdeA binds to Ub in a twisted orientation compared with SENP8 binding to Nedd8 or NopD binding to Ub [25,41,86], caused by the use of a Ub interface surrounding Gln40 instead of the common hydrophobic Ile44 patch. Moreover, OtDUB contains multiple Ub-binding domains, favoring the recognition of longer Ub chains [40]. OtDUB is a remarkable example of VR plasticity, along with the viral protease from ASFV [40,54]. OtDUB lacks the VR-1, but its C-terminal accessory domains extend into the VR-1 location and assist substrate binding. ASFV also has distinct VR-1 and VR-2 regions, with an additional loop inserted in VR-3.

Broad-range specificity in bacterial CE-clan proteases

The broad-range activity exhibited by some CE-clan proteases in bacteria exemplifies the remarkable plasticity of the catalytic domain for substrate recognition. Proteases such as XopD, SdeA, and NopD showcase this versatility. XopD, from the plant pathogenic bacterium *X. campestris*, represents a notable example exhibiting both deSUMOylase and deubiquitinase activities. Through these enzymatic functions, XopD manipulates host cellular processes, particularly transcriptional regulation mediated by host transcription factors [24,38]. Similarly, NopD from *Bradyrhizobium* is an effector protein involved in nodule symbiosis in legumes, with the ability to deconjugate SUMO, Ub, and Nedd8 [41]. Interestingly, both XopD and NopD are effectors proteases from plant pathogens or symbionts that preferentially deconjugate K48-linked Ub chains over K63-linked ones, which contrasts with the preferences of most effector proteins

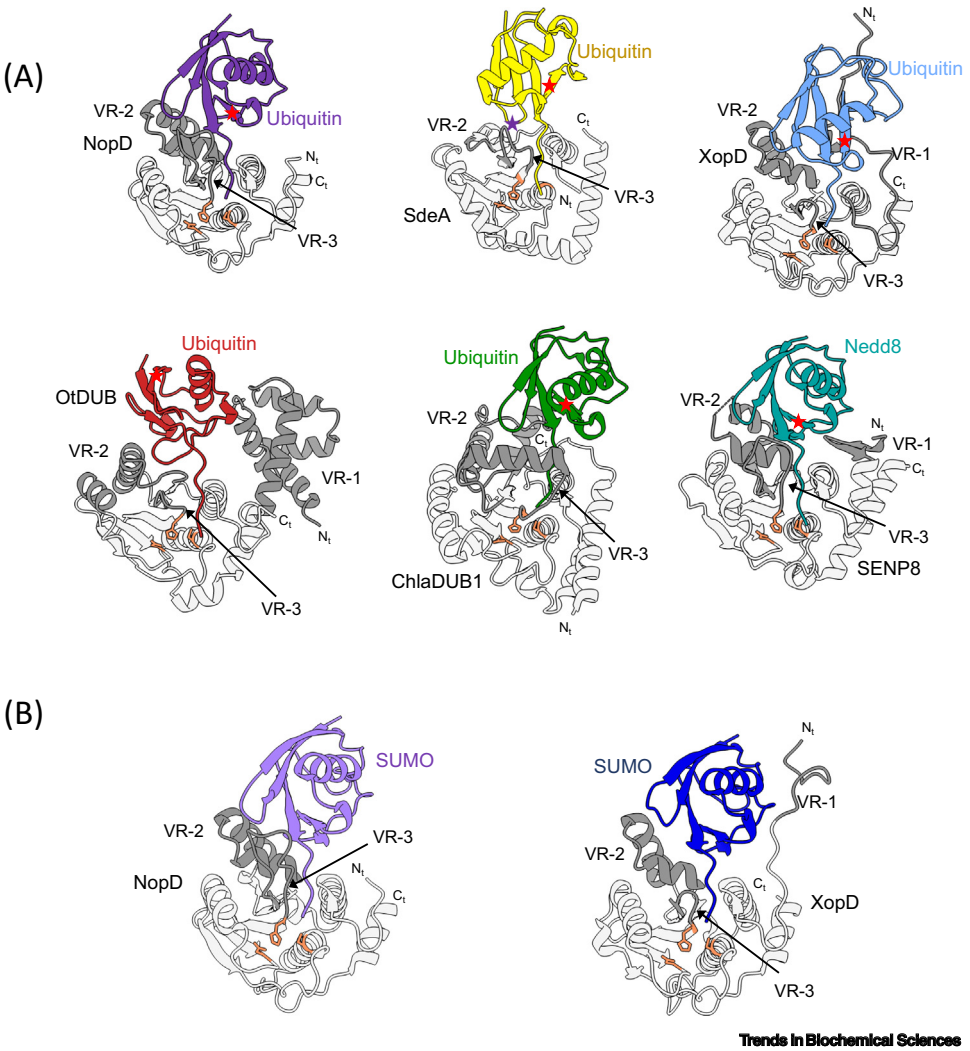

Figure 4. Diverse binding surfaces of ubiquitin (Ub) in complex with members of the CE-clan proteases. (A) Cartoon of NopD-Ub (PDB: 8RQI), SdeA-Ub (PDB: 6WTG), XopD-Ub (PDB: 1JP3), OtDUB-Ub (PDB: 6UPU), ChlaDUB1-Ub (PDB: 6GZS), and SENP8/Den1-Nedd8 (PDB: 1XT9). Ub and neural precursor cell-expressed developmentally downregulated gene 8 (Nedd8) are shown in different colors. The three variable regions (VR-1, VR-2, and VR-3) are indicated and illustrated in gray. The catalytic residues are shown in stick representation and colored orange. Ub Ile44 and Gln70 are illustrated by red and purple stars, respectively. (B) Cartoon of XopD-SUMO (PDB: 5JP1) and NopD-SUMO (PDB: 8OI3). VR-1, VR-2, and VR-3) are indicated and illustrated in gray. The N and C termini are also indicated. The catalytic residues are shown in stick representation and colored orange.

from animal pathogens. SdeA from *L. pneumophila* and a member of the SidE family of effectors cleaves both Ub and Nedd8 with a preference for Ub K63-linked chains [21,24,25].

Recently, SnCE1 from the *Chlamydia-like* pathogen *S. negevensis* was also shown to react with SUMO and Ub *in vitro*, exhibiting specificity for K11- and K48-linked chains. This is unusual because bacterial DUBs typically lack deSUMOylation activity [43]. Other examples of cross-reactivity between Ub and Nedd8 include RickCE and ChlaDUB1 [24,42,48]. Although Ub and Nedd8 share sequence similarity, they are more distantly related to SUMO isoforms. This marked divergence underscores the remarkable adaptability of CE-clan proteases, as exemplified by their ability to engage with both SUMO and Ub.

In XopD, the unstructured VR-1 serves a crucial role in accommodating Ub substrates [24,38]. In the XopD-SUMO complex, the VR-2 region is prominently involved in the interaction, whereas, in the XopD-Ub complex, VR-2 assumes a lesser role, and the interface mainly relies on the unstructured VR-1 segment, forming a novel interface with the hydrophobic patch surrounding Ub Ile44 (Figure 4). SdeA diverges from other CE-clan proteases by using a distinct VR-2 and VR-3 arrangement. Unlike its counterparts, SdeA does not interact with the conserved Ile44 or Arg72 residues on Ub [24,25]. Instead, it engages a novel binding site around Gln40 (Figure 4). This atypical interaction enables SdeA to recognize and interact with Nedd8 and ISG15 in addition to Ub, with a preference for K63-linked polyUb chains. Finally, NopD has a unique VR-3 loop insertion crucial for its broad-spectrum activity by mediating interactions with the C-terminal tail of the target protein. Remarkably, NopD uses the same interface to engage with AtSUMO2, Ub, and Nedd8, a distinctive characteristic among CE-clan proteases given the substantial differences between SUMO and Ub surfaces [41].

These examples collectively highlight the plasticity of this protease fold, demonstrating its ability to target different UbL modifiers. This versatility contrasts with the typical specificity of eukaryotic CE-clan proteases, which usually target only one type of UbL modifier with strict selectivity.

Concluding remarks

The discovery and characterization of bacterial and viral Ub/UbL proteases represent significant advances in understanding host–pathogen interactions. These enzymes have emerged as key players in infection processes and have become promising targets for combating infections. The adaptability and plasticity of the protease fold underscores the remarkable ability of these enzymes to evolve, highlighting the ability of bacteria and virus to adapt and survive.

One of the intriguing aspects of these proteases is their substrate selectivity, which is determined by variable specific regions within the enzyme. Even subtle changes within these variable regions can lead to dramatic changes in substrate recognition (see Outstanding questions). This selectivity sheds light on the intricate molecular mechanisms underlying their activity. Understanding how these regions collaborate within the context of catalytic efficiency and substrate recognition provides valuable insights into the functioning of these enzymes and their roles in infection processes.

Overall, the increasing characterization of bacterial and viral Ub/UbL proteases not only expands our knowledge of host–pathogen interactions, but also opens new avenues for developing targeted therapies against infections. By elucidating the molecular mechanisms of these enzymes, and how they manipulate the Ub and UbL code, better strategies can be designed to disrupt their activity and combat infections.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Gemini, a language model from Goggle AI, in order to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Outstanding questions

How do CE-clan proteases achieve such high substrate specificity despite having a small and conserved fold?

What are the molecular mechanisms by which bacterial and viral proteases disrupt host Ub and UbL pathways?

What are the functional implications of the structural differences between pathogenic bacterial CE proteases and their eukaryotic counterparts?

Does the unstructured N-terminal region of proteases participate in substrate recognition and specificity?

What evolutionary pressures have driven the development of dual or multiple substrate specificities in bacterial and viral CE-clan proteases?

What roles do CE-clan proteases have in the lifecycle and pathogenicity of viruses such as ASFV and mpox virus?

Are protease inhibitors targeting CE-clan effectors a promising strategy for combating bacterial and viral infections?

Declaration of interests

The authors declare no competing interests.

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