Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Chapter 494

Coronavirus Papain-like Peptidases

Databanks

MEROPS name: murine hepatitis coronavirus papain-like peptidase 1
MEROPS classification: clan CA, family C16, subfamily C16A, peptidase C16.001
Species distribution: known only from murine hepatitis virus
Reference sequence from: murine hepatitis virus (UniProt: P19751)

MEROPS name: murine hepatitis coronavirus papain-like peptidase 2
MEROPS classification: clan CA, family C16, subfamily C16B, peptidase C16.006
Species distribution: family Coronaviridae
Reference sequence from: murine hepatitis virus (UniProt: P19751)

MEROPS name: porcine transmissible gastroenteritis coronavirus papain-like peptidase 2
MEROPS classification: clan CA, family C16, subfamily C16B, peptidase C16.008
Species distribution: known only from transmissible gastroenteritis virus
Reference sequence from: transmissible gastroenteritis virus

MEROPS name: SARS coronavirus papain-like peptidase
MEROPS classification: clan CA, family C16, subfamily C16B, peptidase C16.009
Tertiary structure: Available
Species distribution: family Coronaviridae
Reference sequence from: SARS coronavirus (UniProt: P59641)

MEROPS name: human coronavirus 229E papain-like peptidase 2
MEROPS classification: clan CA, family C16, subfamily C16B, peptidase C16.010
Species distribution: known only from human coronavirus
Reference sequence from: human coronavirus (UniProt: Q05002)

Name and History

Proteolytic processing of a polyprotein precursor is an event common to the replication cycle of many RNA viruses. For coronaviruses, a family of positive-stranded RNA viruses with large genomes (28–32 kb), the gene encoding the viral non-structural proteins (nsp's), including the RNA-dependent RNA-polymerase, is translated into a large precursor polyprotein, which must be proteolytically processed to mediate viral transcription and replication [1]. Sequence analysis of coronavirus genomic RNA reveals the presence of either 1 or 2 papain-family protease domains that were shown to process the amino-terminal region of the replicase polyprotein (Figure 494.1) [2–8]. The emergence in 2002–2003 of a novel coronavirus with 10% mortality, severe acute respiratory syndrome coronavirus (SARS-CoV), motivated researchers to further characterize the papain-like

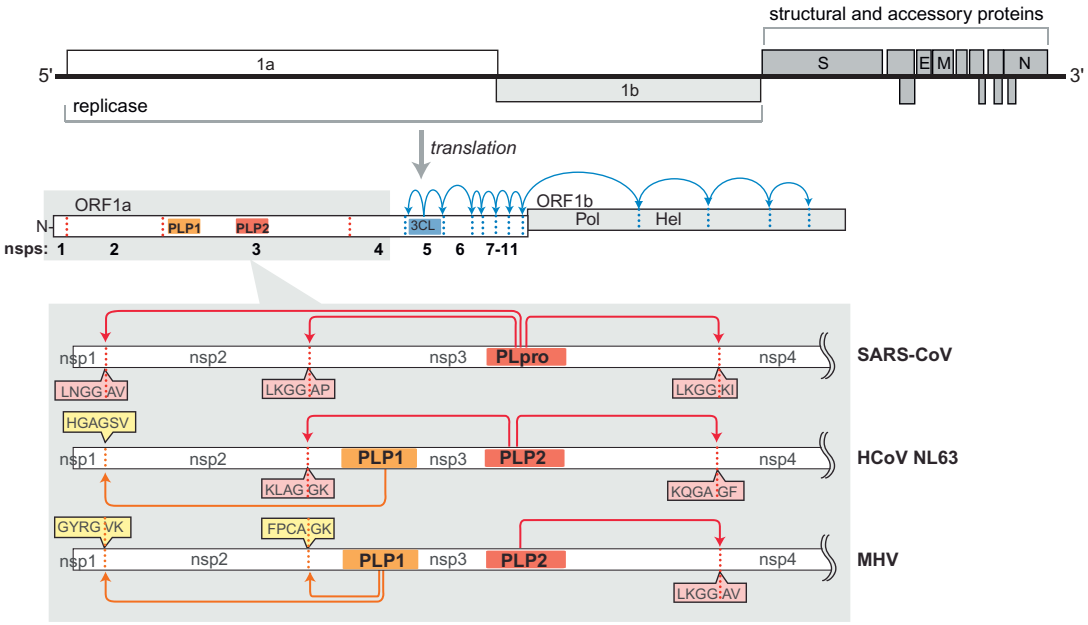

FIGURE 494.1 The amino-terminal end of the coronavirus replicase polyprotein is processed by 1 or 2 papain-like protease domains. Schematic diagram of a coronavirus genome (top) and replicase products (below) generated by translation of the 5′-most open reading frame. The non-structural proteins (nsp's) are generated by the activity of papain-like proteases 1 and 2. Severe acute respiratory syndrome coronavirus (SARS-CoV) encodes only one papain-like protease domain (PLpro) which acts at 3 sites by recognizing the canonical sequence −LXGG. Human coronavirus NL-63 (HCoV-NL63) replicase polyprotein contains 2 papain like protease domains. PLP1 releases nsp1 from the polyprotein and PLP2 processes the nsp2/3 and nsp3/4 sites. The murine coronavirus mouse hepatitis virus (MHV) encodes 2 PLPs with PLP1 processing the nsp1/2 and nsp2/3 sites and PLP2 processing the nsp3/4 junction.

protease domain as a potential target for antiviral drug development [9−11]. These studies validated coronavirus PLP domains as targets for antiviral drugs and revealed the dual nature of coronavirus PLPs as both endopeptidases and isopeptidases. The isopeptidase activity of coronavirus PLPs acts as a deubiquitinating (DUB) and de-ISGylating enzyme that is likely important in viral pathogenesis [12−16].

Structure and Chemistry

Cloning and expression of the N-terminal region of the murine coronavirus replicase polyprotein revealed that a predicted papain-family protease (papain-like protease, PLP) domain was responsible for processing the amino-terminal non-structural protein (nsp) from the replicase polyprotein [2−3]. Further studies revealed the murine coronavirus contained two PLP domains, with PLP1 processing at the nsp1/2 site and the nsp2/3 site [5]. The downstream PLP2 domain processes the nsp3/4 cleavage site using a highly conserved cleavage recognition site of LXGG [17]. Analysis of the N-terminal region of the replicase polyprotein of SARS-CoV revealed only one PLP domain, termed PLpro, which was shown to process the nsp1/2, nsp2/3 and nsp3/4 cleavage sites using the LXGG recognition motif [18]. Interestingly, the PLpro cleavage recognition site, LXGG, is homologous to the LRGG site used by cellular de-ubiquitinating enzymes. Based on this observation, Sulea and colleagues proposed that the SARS-CoV PLpro could have both endopeptidase and isopeptidase activity [19]. This dual substrate recognition and catalytic function of SARS-CoV PLpro was validated independently by two groups [12,13]. These studies showed that a core domain of PLpro could be expressed and purified from *E. coli* and can catalytically process both polyprotein and polyubiquitin substrates. Ultimately, determination of the high resolution X-ray crystal structure of the core domain of SARS-CoV PLpro revealed a canonical Cys-His-Asp catalytic triad within the active site and an adjacent flexible loop, a zinc-finger domain and a ubiquitin-like domain which was not previously predicted as part of the structure (Figure 494.2) [9]. Analysis of the structure revealed homology of SARS-CoV PLpro to cellular de-ubiquitinating enzymes such as USP7 (see Chapter 464) and USP 14 (see Chapter 470). Initially, the similarity between viral and cellular protease structures raised questions about the ability to make an inhibitor that was selective exclusively for the viral protease. High-throughput screening of a modest 50 K compound library and subsequent structure−activity relationship analysis of

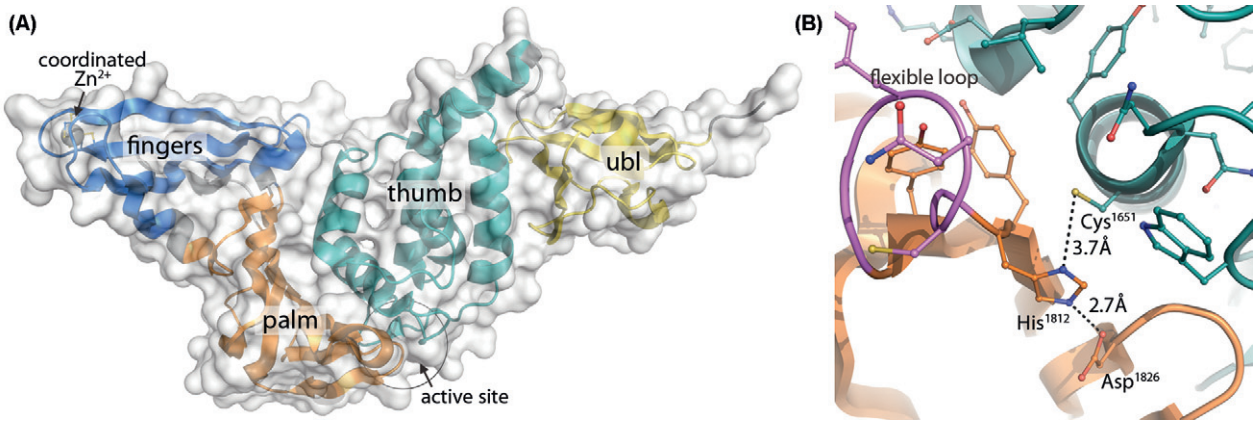

FIGURE 494.2 Structure of severe acute respiratory syndrome papain-like protease domain. (A) The overall structure of the SARS-CoV PLpro domain can be modelled as a left hand, with a fingers domain that coordinates a zinc residue, a palm domain containing the Cys-His-Asp catalytic triad required for endopeptidase and isopeptidase activity, the adjacent thumb domain and the ubiquitin-like domain (ubl) of unknown function; (B) A flexible loop region (purple circle) adjacent to the catalytic triad can be targeted by non-covalent protease inhibitors [10].

lead compounds led to the identification of non-covalent, specific inhibitors of PLpro that also inhibited the replication of SARS-CoV [10,11]. These studies validated SARS-CoV PLpro as a therapeutic target and provided the proof of principle for the development of viral and perhaps even cellular DUB-specific inhibitors.

Activity and Specificity

A soluble and active form of SARS-CoV PLpro was expressed in *E. coli* and purified using column chromatography [12]. This 35 kilodalton protein was evaluated for the ability to process a variety of substrates, including a FRET-based peptide representing polyprotein recognition sequences: E^{Edans}-RELNGG↓APIK^{Dabcyl}-S. To test the de-ubiquitinating activity of the enzyme, PLpro was characterized with several fluorescent, ubiquitin (Ub)-related substrates, including full-length Ub-AMC, ISG15-AMC, and the short peptide RLRGG-AMC, representing the 5 C-terminal residues of ubiquitin and ISG15 [10,12]. All assays were performed at 25°C, in 20 mM HEPES, pH 7.5, 0.1 mg/mL BSA, and 5 mM DTT. With the exception of ISG15-AMC, none of the substrates saturated the enzyme up to the concentrations tested, and therefore pseudo first-order rate constants, k_{app} were reported ($k_{app} \sim k_{cat}/K_m$ for non-saturable enzymes): E^{Edans}-RELNGG↓APIK^{Dabcyl}-S, $0.0244 \pm 0\,0003\ min^{-1}\ \mu M^{-1}$; Ub-AMC, $4.48 \pm 0.1^{-1}\ min^{-1}\ \mu M^{-1}$; RLRGG-AMC, $0.61 \pm 0{\cdot}01\ min^{-1}\ \mu M^{-1}$. PLpro is considerably more active with the ISG15-AMC substrate, producing k_{cat} and K_m values of $370 \pm 16\ min^{-1}$ and $2.3 \pm 0.3\ \mu M$, respectively [10].

Biological Aspects

The coronavirus PLP domains have two major functions: (1) processing of the replicase polyprotein; and (2) antagonism of the innate immune response via de-ubiquitinating and de-ISGlyating target proteins. Coronavirus PLPs play a critical role in the processing of the precursor polyprotein to generate the non-structural proteins associated with viral replication (Figure 494.1). The processed replicase products embed into the endoplasmic reticulum and generate convoluted membranes and double membrane vesicles (DMVs) which are the sites of viral RNA synthesis [1,20,21]. Furthermore, coronavirus PLP isopeptidase activity mediates de-ubiquitination and de-ISGylation of cellular targets, likely blocking the activation of the innate immune response to viral infection [14–16,22,23]. Further studies are needed to determine the role of viral DUB activity in the pathogenesis of coronavirus infections.

Related Peptidases

Either one or two PLP domains have been identified in all coronaviruses sequenced to date (sequences available at www.viperbrc.org). The SARS-CoV PLpro domain is structurally similar to USP7 (see Chapter 464) and USP14 (see chapter 470). In addition, the arteriviruses, which group together with coronaviruses in the order *Nidovirales*, encode functional papain-like cysteine protease domains as described in detail in Chapters 495, 497 and 498.

Further Reading

Recommended papers include those of Ratia *et al*. [9,10], and Perlman & Netland [1].

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