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 499

Nairovirus Deubiquitinylating Peptidase

Databanks

MEROPS name: nairovirus deubiquitinylating peptidase
MEROPS classification: clan CA, family C87, peptidase C87.001
Tertiary structure: Available
Species distribution: known only from Crimean-Congo hemorrhagic fever virus
Reference sequence from: Crimean-Congo hemorrhagic fever virus

Name and History

The ***deubiquitinylating peptidases*** (***DUBs***) remove or deconjugate ubiquitin chains from ubiquitinated proteins [1]. There are several different classes of DUBs, one of them belongs to the ovarian tumor (OTU) domain-containing family of proteins [2], which comprises a group of cysteine proteases homologous to the OTU protein of Drosophila [3]. The OTU domain-containing protein family includes more than a hundred proteins from eukaryotes, bacteria, and viruses [4]. Recently, it was found that the amino-terminal region of the RNA-dependent RNA polymerase, known as L protein, of nairoviruses, including Crimean-Congo hemorrhagic fever virus (CCHFV) and Dugbe virus, contains an OTU domain based in homology with other OTU domain-containing proteins (Figure 499.1) [5,6].

Nairoviruses (genus *Nairovirus)* are negative strand, segmented RNA viruses from the *Bunyaviridae* family. These viruses are the etiological agents of several types of hemorrhagic fever syndromes with high mortality in regions of Asia, Europe, and Africa [7,8]. Nairoviruses must defeat the antiviral and inflammatory effects of innate immune cytokines such as type I interferon and tumor necrosis factor alpha, in order to replicate in mammalian hosts. Both the induction and the effects of antiviral cytokines are controlled by Ub (Ubiquitin) and Ub-like (UBL) molecules. An interesting example is the interferon-stimulated gene 15 (ISG15) system, responsible for the conjugation of ISG15, a UBL molecule, onto target proteins, resulting in antiviral effects [9].

Frias-Staheli *et al*. [6] demonstrated that the CCHFV L protein has deconjugating activity associated with its OTU domain, but that in contrast to mammalian OTU-domain containing DUBs, which appear to only deconjugate Ub, the CCHFV-OTU domain deconjugates both Ub and ISG15 (Figure 499.2). The OTU protease activity of CCHFV L is not necessary for its RNA polymerase activity [10]. Rather, the deconjugating activity of CCHFV L disarms antiviral innate processes regulated by ubiquitination and ISGylation, such as the induction of nuclear factor kappa B (NF-κB) and the ISG15 antiviral activity [6]. Recently, the expression of the OTU domain of CCHFV in *E. coli* by three independent groups has permitted the enzymatic characterization of this protease and also allowed the determination of its structure [11–13].

Activity and Specificity

The nairovirus OTU domain proteases have demonstrated the capacity to cleave a wide broad spectrum of Ub and UBL conjugates, including ISG15 conjugates. However, not all UBL conjugates, for example SUMO conjugates, are cleaved by this protease. The sequence recognized by nairoviruses OTU proteases includes the C-terminal LRLRGG segment present both in Ub and in ISG15, but not in SUMO. These six amino acids interact with OTU amino acid residues at or in the surrounding area of the OTU catalytic site [11–13].

Most of the characterization of the enzymatic properties of the OTU domain of nairoviruses has been performed with CCHFV-OTU, corresponding to the approximately first 170 amino acids of the CCHFV L protein. Activity can be determined by co-expression in 293T cells of CCHFV-OTU with either HA tagged-Ub or His-tagged ISG15 and their conjugating enzymes, UBE1L and Ubc8. Total protein conjugation can be visualized by immunoblotting with anti-HA or anti-His antibodies (Figure 499.2) [6].

The enzymatic activity of CCHFV-OTU can also be studied in a cell-free system, based in the incubation of bacterially expressed and purified CCHFV-OTU protease with Ub-AMC, ISG15-AMC, RLRGG-AMC, Ub2 K63 FiAsh or NEDD8-AMC substrates (Table 499.1).

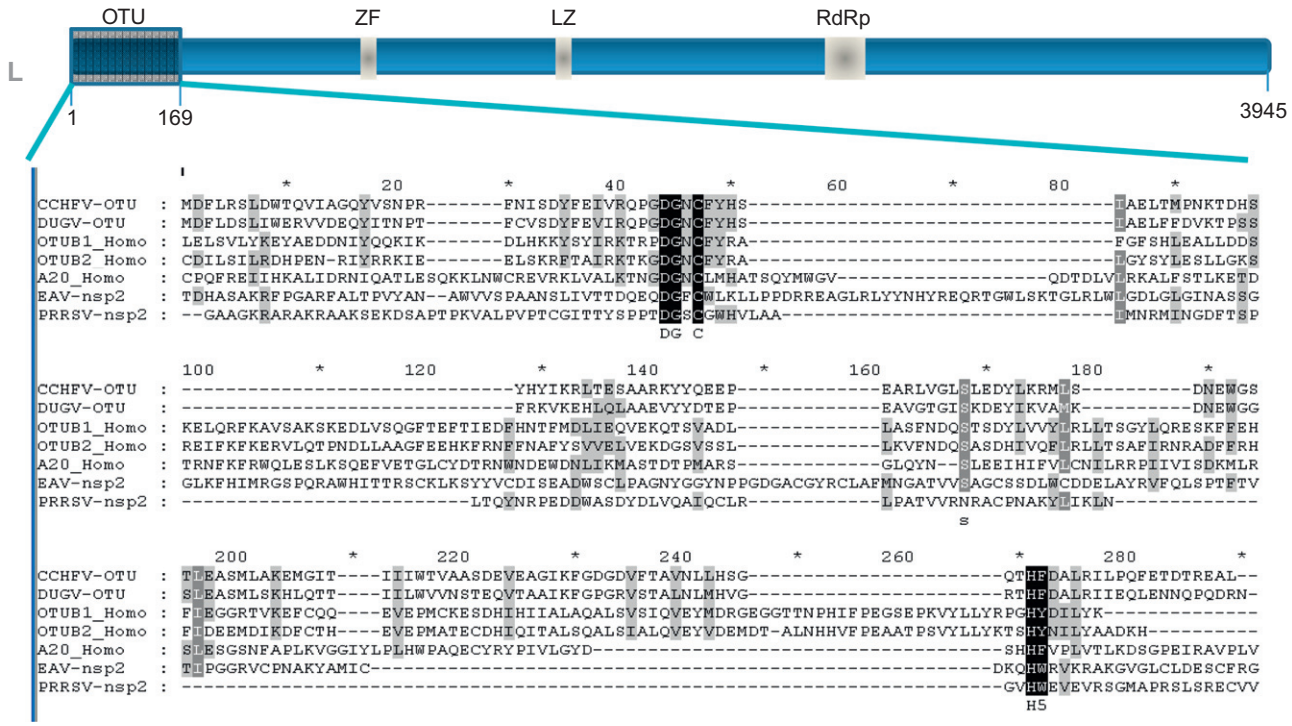

FIGURE 499.1 Multiple sequence alignment of representative OTU domains. Strictly conserved amino acids are marked in black, highly conserved residues are shaded in gray. The GenBank accession numbers of the OTU domain sequences used are as follows: Nairoviruses CCHFV-OTU (AAY24690.1), and DUGV-OTU (AAB18834.1, Dugbe virus). Arteriviruses EAV-nsp2 (residues 120−352 of CAC42774, Equine arteritis virus), and PRRSV-nsp2 (residues 385−518 of AAT95214, Porcine respiratory and reproductive syndrome virus). Human proteins A20-Homo sapiens (residues 57−277 ACN87232), OTUB1-*Homo sapiens* (residues 52−271 of NP_060140, Otubain 1) and OTUB2-*Homo sapiens* (residues 13−234 of NP_075601 Otubain 2). A schematic representation of the 3945 amino acid long L protein of nairoviruses is shown in the top, highlighting the OTU domain, and ZF (zinc finger), LZ (leucine zipper) and RdRp (RNA dependent RNA polymerase) domains.

Assay Methods

Unpublished data from our group reveal that the optimal pH of the enzyme is around 8, but lower pHs, such as pH 7.4, have also been used with good results [13]. The redox conditions are very important for the optimal activity of the enzyme, 2 mM DTT is recommended as the minimal concentration of reducing agent to be added to the reaction buffer. These conditions are necessary for the reduction of the cysteine in the active site (corresponding to Cys40 in CCHFV-OTU).

Multiple experiments have been done to elucidate the CCHFV-OTU's deubiquitinating activity towards K63- and K48-linked poly-Ub. The results indicate that there are no major differences in CCHFV-OTU's activity towards the two types of Ub linkage. The CCHFV-OTU enzymatic activity was markedly decreased by mutating amino acid Cys40, indicating that this residue is required for optimal protease activity [6]. Atkin *et al.* [12] showed that CCHFV-OTU cleaves also K6- and K11-Ub linkages, but it is not able to cleave K29-linked and linear diUb.

CCHFV-OTU is inhibited by general inhibitors of cysteine proteases, such as sulfhydryl alkylating agent NEM and the peptide aldehyde antipain [14]. Also as expected, CCHFV-OTU is inhibited by specific inhibitors of DUB enzymes corresponding to ubiquitin and ISG15 derivates coupled to aldehyde or vinyl sulfone [15−18]. So far there have been no reports of specific inhibitors for the *nairovirus* proteases.

Structural Chemistry

Primary Structure

The CCHFV active OTU domain corresponds to amino acid residues 1 to 169 of the 3945 amino acid long L protein. There are no known potential covalent modifications of these viral proteases. Bioinformatics predictions features reported by the ELM resource, or the software Motif Scan, detect one potential CK1 phosphorylation site at amino acid residues SGQTHFD 147−153 and three potential CK2 phosphorylation sites at FYHSIAE 41−47, RMLSDNE 92−98 and EWGSTLE 98−104. No metal atoms were described in any of the seven X-ray structures described so far [11−13].

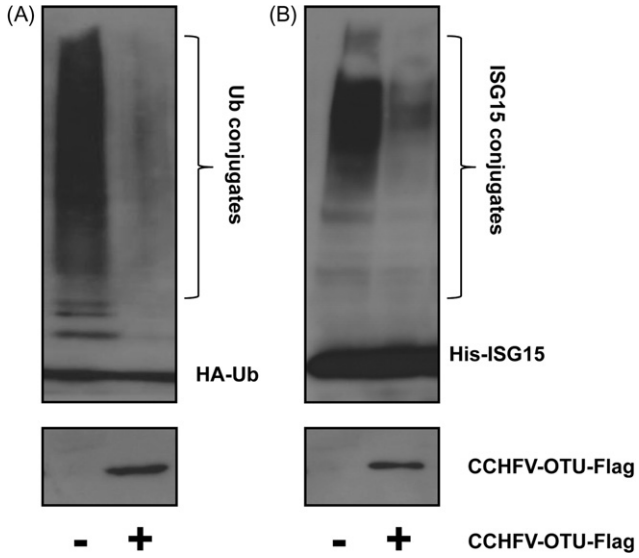

FIGURE 499.2 Decreasing levels of Ub and ISG15 conjugates in cells expressing CCHFV-OTU protein. 293T were transfected with the plasmids expressing either HA-Ub (panel A) or His-ISG15, UBE1L and Ubc8 (panel B) along with Flag-tagged CCHFV-OTU. Total protein ubiquitination was visualized by immunoblotting with anti-HA (panel A) and protein ISGylation was visualized by anti-His immunoblot (panel B). These samples were also probed with Flag for detection of the CCHFV-OTU tagged protein.

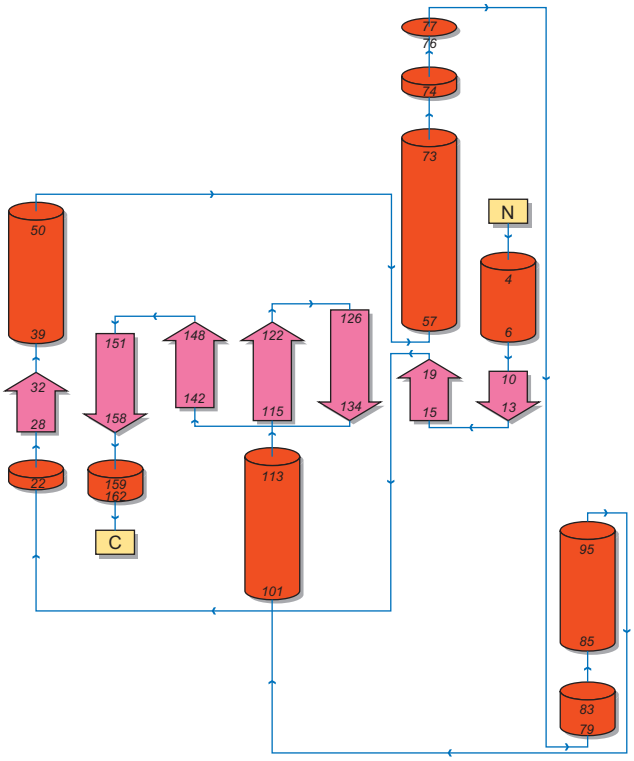

FIGURE 499.3 CCHFV-OTU protease topology based on the X-ray structure 3PT2. Topology obtained with the program PROMITIF. The α-helix structures are represented in red cylinders and the β-sheets in pink arrows. The N- and C-terminals are represented with yellow boxes.

TABLE 499.1 Kinetic parameters for cleavage of various UBL conjugates

Substrate	K_m (μM)	k_{cat}/K_m (M^{-1} s^{-1})	References
Ub-AMC	14.2	3.41×10^5	[11]
ISG15-AMC	4.7	3.6×10^5	
RLRGG-AMC	100.7	0.6	
Ub-AMC	12.8	2.9×10^5	[12]
ISG15-AMC	5.5	1.4×10^5	
Ub2 K63 FiAsh	0.93		
Ub-AMC	3.8		[13]
ISG15	2.2		
NEDD8-AMC	26.6		

Secondary Structure

The CCHFV-OTU protease secondary structure has been clearly established by the recently published X-ray structures [11–13]. The analysis of the structure PDB code: 3PT2 (total residues 164) [11] characterizes this protease as having seven stranded β-sheets (24.4%) and nine α helices (33.5%) (Figures 499.3 and 499.4). Remarkably, all the structures obtained so far of CCHFV-OTU have identified unique N-terminal β-strand elements that allow binding to both Ub and ISG15 substrates, as compared with previously described OTU peptidases that only deconjugate Ub [11–13]. The CCHFV-OTU X-ray structure in complex with Ub revealed that the N-terminal β-strands interact with a hydrophobic patch in Ub, resulting in a −75° rotation of Ub with relationship to the OTU protease, as compared to OTUB1-Ub complex [11]. On the other hand, ISG15 interacts by two hydrophobic surfaces with complementary surfaces on the CCHFV-OTU protease, resulting in higher affinity of the protease for ISG15 than for Ub (reflected by a 2-fold lower K_m). Nevertheless, the viral protease is slightly more active against Ub, as demonstrated by the k_{cat} constant [12].

Tertiary Structure

The CCHFV-OTU protease consists of two lobes that clamp around a central substrate-binding furrow, finishing in a channel where C-terminal of Ub or ISG15 is directed towards the enzyme active site (Figure 499.5). As

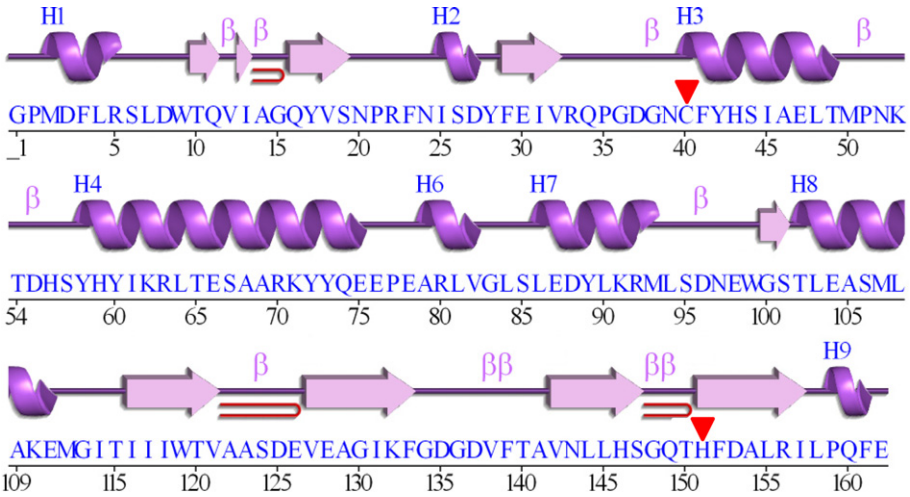

FIGURE 499.4 CCHFV-OTU protease secondary structure based on the X-ray structure 3PT2. α-Helices are represented in dark purple (labeling below), β-strands in light purple, the β turns with the letter β and the β hairpins with a red hairpin. Also, residues in the active sites are marked with red arrow heads.

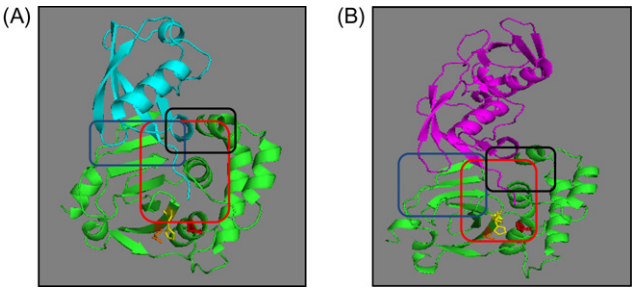

FIGURE 499.5 Crystal structures of CCHFV-OTU (green) bound to Ub (cyan) or ISG15 (magenta). Cartoon schemes of the complex highlighting the three major regions of interaction between Ub (A) or ISG15 (B) and the protease. The nucleophile (Cys40) is indicated in red, located on helix α3, His151 is indicated in yellow and Asp153 in orange. Figures were drawn using PyMol [28]. Regions 1, 2 and 3 are indicates with red, black and blue squares, respectively.

described by James *et al*. [11], the larger of the two lobes (amino acids 1−34 and 114−162) is composed of seven-stranded β-sheets (β1↑ β2↓ β3↑ β4↓ β5↓ β6↑ β7↓) sandwiched between helices α1, α2, α3, and α7. Opposed, the smaller lobe (residues 35−113) is exclusively α-helical (composed of helices α3, α4, α5, α6, and α7). The interaction with both Ub and ISG15 is localized in three regions: the first region in characterized by the channel that guides the C-terminal motifs of Ub or ISG15 towards the enzyme active site, region 2 is located on the α-helical lobe and region 3 in the β-sheet lobe. In this last region where the protease generates hydrophobic interactions with Ub and electrostatic interactions with ISG15, and together with the region 2, forms the interface that binds the β-grasp folds of Ub or of the C-terminal of ISG15 [11]. It should be noted that ISG15 structurally resembles two molecules of Ub.

In the active site, the cysteine thiol must be deprotonated by His151 to promote a nucleophilic attack by the Cys40 on the carbonyl carbon of the scissile peptide bond of the target substrate; this permits the formation of a covalent acyl-enzyme intermediate, which is resolved by a water molecule [19]. The carboxyl group of Asp153 in CCHFV1-OTU enhances catalysis of the viral protease by hydrogen-bonding with the imidazole group of His151 [11].

Quaternary Structure

The calculated pI of the enzyme is 4.79 and the calculated molecular mass of a monomer is 19306.69 Da. The oligomeric state of CCHFV-OTU-Ub complex and CCHFV-OTU structures has been investigated using static light scattering. For CCHFV-OTU, 95.7% of the sample's molecular mass was calculated at 18.7 kDa, denoting that CCHFV-OTU has a monomeric form in solution. As for CCHFV-OTU-Ub molecules, 72.5% of the sample possessed a monomeric complex molecular mass of 27.5 kDa. The 25% of the sample acquired a molecular weight of 54.2 kDa. These results propose that the most feasible biologic assembly is one CCHFV-OTU protein interacting with one Ub molecule [13].

Preparation

Frias-Staheli *et al*. 2007 [6] generated a CCHFV-OTU bacterial expression plasmid by incorporating the coding sequence of the first 169 residues of CCHFV L (GenBank accession no. AAQ98866.2) into pET11a plasmid using NdeI and BamHI restriction sites. Six His codons were added to the sequence to provide a C-terminus

purification tag. Atkin *et al*. [12] generated a CCHFV-OTU bacterial expression plasmid by cloning the coding sequence of the first 217 residues of CCHFV L into the pOPIN-K vector that incorporates a glutathione *S*-transferase (GST) tag. The GST tag is cleaved with GST PreScission Protease, with subsequent purification by anion-exchange chromatography (RESOURCE Q, GE Life Sciences), followed by gel filtration chromatography (HiLoad 16/60 Superdex 75 column, GE Life Sciences) [12].

In order to express CCHFV-OTU in mammalian cells, one recommended vector is the pCAGGS plasmid which expresses transcripts under the control of a chicken β-actin promoter [20]. As described by Frias-Staheli *et al*. [6], the multiple cloning site of this original vector was modified in order to facilitate the cloning strategy generating a pCAGGS-MCSII vector. N-terminal or C-terminal HA tags can be introduced into the construct by PCR with gene-specific primers possessing the tag sequence. Same strategy has been used to express the Dugbe virus OTU domain in mammalian cells [6].

Biological Aspects

The ORF of the CCHFV-OTU is expressed as part of the N-terminus of the L protein of CCHFV, encoded by the L RNA segment of this virus. It is naturally expressed using the known transcriptional strategies of negative strand RNA viruses: the UTRs of the viral RNAs contain all of the *cis*-acting elements necessary for virus transcription by the viral RNA polymerase [21].

The cytoplasmic replication cycle of the *bunyaviruses* implies that the L protein of *nairoviruses* is localized in the host cell cytoplasm; this has been proved by immunofluorescence microscopy by Frias-Staheli *et al*. [6] and by Bergeron *et al*. [10]. Also tagging the CCHFV-OTU protein with GFP allowed it to directly localize in the cytoplasm (Figure 499.6).

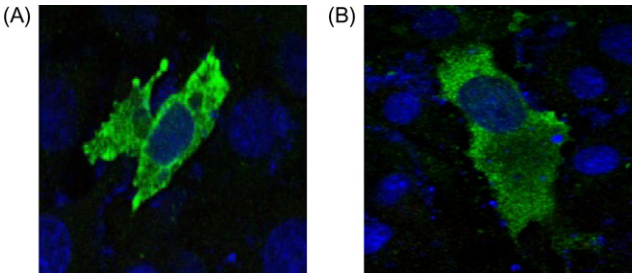

FIGURE 499.6 CCHFV-OTT GFP localizes in the cytoplasm. 293T (A) or BSR (B) cells were transfected with the plasmid expressing the fusion protein CCHFV-OTU GFP. Nuclei were stained with DAPI (blue).

No proenzyme activation has been shown to be necessary for the DUB action of CCHFV-OTU. However, it remains to be determined if other structural components of the viral L protein can modulate the DUB and deISGylating activities of the CCHFV-OTU domain during viral replication.

The nairovirus proteases have been described in CCHFV, DUB and Nairobi sheep disease (NSD) [5,6], but is also found in the sequence of the L protein of the other 34 members of the genus, like Hazara or Ganjam viruses [22].

Interestingly, the double capacity of cleavage Ub and ISG15 from conjugated target proteins by the CCHFV-OTU seems to be an evolutionary adaptation to suppress the innate immune response related to these two protein ligands. Moreover, viral OTU domains negatively regulate the NF-kB pathway [6].

The interferon pathway plays a crucial role in the innate immune response against viral infections. One of the mechanisms that *nairoviruses* appear to have developed to circumvent this response is the proteolytic cleavage of Ub and of Ubl, like ISG15 [23]. Due to the biosafety level 4 required to handle CCHFV, the physiological importance of OTU proteases in the disease involvement has not been directly studied. However, Frias-Staheli *et al*. [6], via the use of transgenic mice, recombinant chimeric Sindbis viruses and transfected cells, have demonstrated that the viral OTU domain proteases have inhibitory effects on Ub- and ISG15-dependent host processes leading to antiviral innate immunity. Therefore, these proteins have bona fide immune evasion properties.

Distinguishing Features

The nairovirus peptidases have both DUB and deISGylase activities. Quantitative kinetic measurements revealed a slightly higher affinity of CCHFV-OTU for ISG15 instead of Ub. The CCHFV-OTU protein cleaves four out of six tested chain types of poly-Ub molecules, including K6-linkages that other OTU DUBs fail to hydrolyze, but is inefficient in cleaving linear and K29-linked chains.

Antibodies

There are not commercially available antibodies against CCHFV-OTU protein. The tagged HA, Flag, HIS, GFP or GST recombinant proteins are useful to detect expression, and no change in the activity has been observed between these recombinant proteins and the untagged protein.

Related Peptidases

Arterivirus nsp2 proteases are also viral OTU proteases with the ability to deconjugate both Ub and ISG15 [24]. In contrast to the nairoviruses OTU proteases, the arterivirus nsp2 proteases participate in the processing of the viral polymerases. At the moment, no crystal structure is available for these proteases. It is also worthy to mention that other viral peptidases, as the adenoviral protease adenain [25] and the papain-like protease from SARS coronavirus [26,27] have ISG15 and Ub deconjugating activities, which have been suggested to suppress inflammatory and IFN responses of the host.

Further Reading

Frias-Staheli *et al.* [6] describe the activity of CCHFV-OTU protease, the role in the repression of the innate immune response, and a functional comparative analysis with other OTU proteases. Versteeg & García-Sastre [23] is an interesting review which offers a graphic overview of the antiviral strategies on the development of the virus. Websites for The International Committee on Taxonomy of Viruses provides information on the molecular biology of different viruses; and Boston Biochem provides information on the reagents for the enzymatic assays described before. Finally, EMBL-MBI is a very useful website to make structural analyses of the proteins.

References

[1] Komander, D., Clague, M.J., Urbé, S. (2009). Breaking the chains: structure and function of the deubiquitinases. *Nat. Rev. Mol. Cell Biol.* 10(8), 550–563.

[2] Balakirev, M.Y., Tcherniuk, S.O., Jaquinod, M., Chroboczek, J. (2003). Otubains: a new family of cysteine proteases in the ubiquitin pathway. *EMBO Rep.* 4, 517–522.

[3] Tirronen, M., Partanen, M., Heino, T.O., Heino, T.I., Roos, C. (1993). Analyses of the Drosophila quit, ovarian tumor and shut down mutants in oocyte differentiation using in situ hybridization. *Mech. Dev.* 40(1–2), 113–126.

[4] Makarova, K.S., Aravind, L., Koonin, E.V. (2000). A novel superfamily of predicted cysteine proteases from eukaryotes, viruses and Chlamydia pneumoniae. *Trends Biochem. Sci.* 25, 50–52.

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

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

[7] Nichol, S.T. (2001). Bunyaviridae, in: *Fields Virology*, 4th edn, Knipe, D.M., Howley, P., eds. Philadelphia: Lippincott, Williams and Wilkins, pp. 1603–1633.

[8] Swanepoel, R. (1994). *Crimean-Congo Haemorrhagic Fever*, Cape Town, South Africa: Oxford University Press, 723–729.

[9] Kirkin, V., Dikic, I. (2007). Role of ubiquitin- and Ubl-binding proteins in cell signaling. *Curr. Opin. Cell Biol.* 19(2), 199–205.

[10] Bergeron, E., Albarino, C.G., Khristova, M.L., Nichol, S.T. (2009). Crimean-Congo hemorrhagic fever virus encoded ovarian tumor protease activity is dispensable for virus RNA polymerase function. *J. Virol.* 84, 216–222.

[11] James, T.W., Frias-Staheli, N., Bacik, J.P., Levingston Macleod, J.M., Khajehpour, M., García-Sastre, A., Mark, B.L. (2011). Structural basis for the removal of ubiquitin and interferon-stimulated gene 15 by a viral ovarian tumor domain-containing protease. *Proc. Natl. Acad. Sci.* 108(6), 2222–2227.

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

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

[14] Nicholson, B., Leach, C.A., Goldenberg, S.J., Francis, D.M., Kodrasov, M.P., Tian, X., Shanks, J., Sterner, D.E., Bernal, A., Mattern, M.R., Wilkinson, K.D., Butt, T.R. (2008). Characterization of ubiquitin and ubiquitin-like-protein isopeptidase activities. *Protein Sci.* 17(6), 1035–1043.

[15] Hershko, A., Rose, I.A. (1987). Ubiquitin-aldehyde: a general inhibitor of ubiquitin-recycling processes. *Proc. Natl. Acad. Sci.* 84, 1829–1833.

[16] Galardy, P., Ploegh, H.L., Ovaa, H. (2005). Mechanism-based proteomics tools based on ubiquitin and ubiquitin-like proteins: crystallography, activity profiling, and protease identification. *Methods Enzymol.* 399, 120–131.

[17] Reverter, D., Wu, K., Erdene, T.G., Pan, Z.Q., Wilkinson, K.D., Lima, C.D. (2005). Structure of a complex between Nedd8 and the Ulp/Senp protease family member Den1. *J. Mol. Biol.* 345(1), 141–151.

[18] Borodovsky, A., Ovaa, H., Meester, W.J., Venanzi, E.S., Bogyo, M.S., Hekking, B.G., Ploegh, H.L., Kessler, B.M., Overkleeft, H.S. (2005). Small-molecule inhibitors and probes for ubiquitin- and ubiquitin-like-specific proteases. *Chembiochem.* 6(2), 287–291.

[19] Rawlings, N.D., Barrett, A.J., Bateman, A. (2010). MEROPS: The peptidase database. *Nucleic Acids Res.* 38(Suppl. 1), D227–233.

[20] Niwa, M., Berget, S.M. (1991). Polyadenylation precedes splicing *in vitro*. *Gene Expression* 1(1), 5–14.

[21] Schmaljohn, C.S. (1996). Bunyaviridae: the viruses and their replication, in: *Fields Virology*, Fields, B.N., Knipe, D.M., Howley, P. M., eds. Lippincott-Raven Publishers, pp. 1447–1471.

[22] Marriott, A.C., Ward, V.K., Higgs, S., Nuttall, P.A. (1990). RNA probes detect nucleotide sequence homology between members of two different nairovirus serogroups. *Virus Res.* 16, 77–81.

[23] Versteeg, G.J., García-Sastre, A. (2010). Viral tricks to grid-lock the type I interferon system. *Current Opinion in Microbiology* 13(4), 508–516.

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

[25] Balakirev, M.Y., Jaquinod, M., Haas, A.L., Chroboczek, J. (2002). Deubiquitinating function of adenovirus proteinase. *J. Virol.* 76, 6323–6331.

[26] Lindner, H.A., Fotouhi-Ardakani, N., Lytvyn, V., Lachance, P., Sulea, T., Ménard, R. (2005). The papain-like protease from the severe acute respiratory syndrome coronavirus is a deubiquitinating enzyme. *J. Virol.* 79, 15199–15208.

[27] Clementz, M.A., Chen, Z., Banach, B.S., Wang, Y., Sun, L., Ratia, K., Baez-Santos, Y.M, Wang, J., Takayama, J., Ghosh, A.K., Li, K., Mesecar, A.D., Baker, S.C. (2010). Deubiquitinating and interferon antagonism activities of coronavirus papain-like proteases. *J. Virol.* 84, 4619–4629.

[28] DeLano, W.L. (2002). The PyMOL Molecular Graphics System (DeLano Scientific, PaloAlto).

Adolfo García-Sastre

Department of Microbiology, Department of Medicine, Division of Infectious Diseases and Global Health and Emerging Pathogens Institute, Mount Sinai School of Medicine, 1468 Madison Avenue, NY 10029, New York, USA.

Jesica M. Levingston Macleod

Department of Microbiology, and Global Health and Emerging Pathogens Institute, Mount Sinai School of Medicine, 1468 Madison Avenue, NY 10029, New York, USA.

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