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Molecular modeling and analysis of hepatitis E virus (HEV) papain-like cysteine protease

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The biochemical or biophysical characterization of a papain-like cysteine protease in HEV ORF1-encoded polyprotein still remains elusive. Very recently, we have demonstrated the indispensability of ORF1 protease-domain cysteines and histidines in HEV replication, ex vivo (Parvez, 2013). In this report, the polyprotein partial sequences of HEV strains and genetically-related RNA viruses were analyzed, in silico. Employing the consensus-prediction results of RUBV-p150 protease as structural-template, a 3D model of HEV-protease was deduced. Similar to RUBV-p150, a ‘papain-like β-barrel fold’ structurally confirmed the classification of HEV-protease. Further, we recognized a catalytic ‘Cys434-His443’ dyad homologue of RUBV-p150 (Cys1152-His1273) and FMDV-L106 (Cys51-His148) in line with our previous mutational analysis that showed essentiality of ‘His443’ but not ‘His590’ in HEV viability. Moreover, a RUBV ‘Zn²⁺ binding motif’ (Cys1167-Cys1175-Cys1178-Cys1225-Cys1227) equivalent of HEV was identified as ‘Cys457-His458-Cys459 and Cys481-Cys483’ residues within the β-barrel fold. Notably, unlike RUBV, ‘His458’ also clustered therein, that was in conformity with the consensus cysteine protease Zn²⁺-binding motif. By homology, we also proposed an overlapping ‘Ca²⁺-binding site’ ‘D-X-DNX-X1/2X5X1/2-X2-DE’ signature, and a ‘proline-rich motif’ interacting ‘tryptophan (W437-W472)’ module in the modeled structure. Our analysis of the predicted model therefore, warrants critical roles of the ‘catalytic dyad’ and ‘divalent metal-binding motifs’ in HEV protease structural-integrity, ORF1 self-processing, and RNA replication. This however, needs further experimental validations.

1. Introduction

Proteases or proteinases are proteolytic enzymes that regulate biological processes in cellular organisms and viruses. Proteases are classified according to their catalytic site into four major classes: cysteine (thiol) proteases, serine proteases, aspartyl (acid) proteases, and metalloproteases. Of the 21 families of cysteine proteases discovered so far, almost half of them are coded by viruses (Grzonka et al., 2001). The best characterized family member of cysteine proteases is the ‘papain’ family characterized by a two-domain structure that contains an active site (catalytic pocket) for substrate binding. Some RNA virus-encoded papain-like cysteine proteases with Cysteine(Cys)-Histidine(His)-Aspartate(Asp) catalytic triads employ a structural zinc²⁺-binding motif residing between the loops of the two β-barrel folds (Sommergruber et al., 1997; Yu and Lloyd, 1992). However, the foot-and-mouth disease virus (FMDV) leader protease (L₁⁰⁵), one of the most well-characterized viral papain-like proteases (Ryan and Flint, 1997), including the rubella virus (RUBV) p150 (Chen et al., 1996) and the human coronavirus-229E (HCoV-229E) PL₁₁₀p (Herold et al., 1998) has shown a ‘Cys-His’ catalytic ‘dyad’ instead of a ‘triad’. The RNA-interacting zinc²⁺-binding proteases are critical in virus replications that include ULS52 of herpes simplex virus type 1 (HSV-1) (Chen et al., 2005), the nucleocapsids of human immunodeficiency virus (HIV-1) and murine-leukaemia virus (MLV) (Gorelick et al., 1996; Mark-Danielli et al., 2005), the V-protein of Sendai virus (SENV) (Fukuhara et al., 2002), and the NSA of hepatitis C virus (HCV) (Tellinghuisen et al., 2004). Of the several different ‘Ca²⁺-binding motifs’, the most common is a ‘helix-loop-helix’ structure referred to as the ‘EF-hand’. Viral ‘EF-hand Ca²⁺-binding motifs’ are also reported in rotavirus VP7, HIV-1-gp41 and polyomavirus-VP1 structural proteins, including the RUBV-nonstructural p₁⁵₀ (Zhou et al., 2007).

Hepatitis E virus (HEV), the etiological agent of acute and chronic hepatitis E, is a non-enveloped positive-sense RNA virus (Holla et al., 2013; Kamar et al., 2012). The HEV open reading frame 1 (ORF1) codes for nonstructural polyprotein (~185 kDa) wherein the validity of a papain-like cysteine protease-domain (a.a. 434–590) (Fig. 1A) still remains a bottle-neck in the understanding of its biology. Also, the expression and purification of HEV-protease and therefore, its biochemical or biophysical characterization is not

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achieved, so far. Nevertheless, there are ample convincing molecular evidences, showing ORF1 polyprotein processing (Parvez, 2013; Karpe and Lole, 2011; Selgul et al., 2006; Kopp et al., 2000; Ansari et al., 2000) along with a few conflicting reports (Perttilä et al., 2013; Suppliah et al., 2011). Very recently, using HEV-SAR55 replicon-system, the indispensability of ORF1 protease-domain ‘catalytic’ as well as X-domain ‘protease-substrate’ residues in HEV replication in hepatoma S10-3 cells, is demonstrated (Parvez, 2013). With this supporting information and the known biology of genetically-close RNA virus proteases, we proposed a three-dimensional (3D) structure of HEV-protease using homology modeling.

2. Materials and methods

2.1. Multiple sequence alignment

Protease-domain amino acid (a.a.) sequences of the four recognized HEV genotypes (1, 2, 3 and 4), represented by seventy seven viral strains, including RUBV-p150 and FMDV-Lpro were analyzed, using the programs ClustalW 1.83 with a gap open penalty-10 and gap extension penalty-0.5 (http://embnet.vital-it.ch/software/ClustalW.html) as well as MultAlign (www.multalin.toulouse.inra.fr/multalin).

2.2. Prediction of protein secondary structure

The program, PSIPRED was used to predict the secondary structure of HEV-protease (target) (http://bioinf.cs.ucl.ac.uk/psipred). The PROFsec program was also used to predict secondary structure elements: helix (α, π and 3_10), β-strand (E=extended strand in β-sheet conformation of at least two residues length) and loop (L) in HEV-protease (https://www.predictprotein.org/get_results?req_id=453912). Notably, PROFsec employs a system of neural networks with an expected average accuracy of more than 72% (Rost et al., 2004).

2.3. Homology modeling of HEV-protease

Further, based on the available structural homology of RUBV-p150 (Zhou et al., 2007) with that of FMDV-Lpro (Guare et al., 2000), p50 was used as template for HEV-protease 3D structure modeling using MODELLER v8.2v. Briefly, an alignment of template-target sequences, the atomic coordinates of the template, and a script file served as the input. First, the files of target and template sequences were converted into MODELLER-PDB and the target-template alignment sequences were converted into MODELLER-PDB formats. MODELLER then calculated five 3D models (starting and ending models 1-5) of HEV-protease. The output files in PDB format that contained coordinates of the generated models were viewed in PyMOL 1.6. The final modeled structure validation was performed in PROCHECK v.3.5 (http://nihserver.mbi.ucl.ac.edu/SAVES/). Of the Ramachandran plots obtained using the PDB files of the predicted models, the one showing about 87% residues in the most favored regions, and about 1.3% in the disallowed region, was selected. Also,
for the final model, pseudo-energy profile was generated and Discrete Optimization Protein Energy (DOPE) potential was evaluated.

2.4. Prediction of disulfide-bridge in the modeled structure

Furthermore, formations of disulfide-bridges, if any, between the active cysteine residues in the modeled HEV-protease were predicted using DISULFIND (https://www.predictprotein.org/get_results?req_id=453912).

3. Results

3.1. Conservation of cysteines, histidines and tryptophans in HEV-protease domain

Multiple alignment-based consensus sequences that contained highly conserved cysteine, histidine and tryptophan residues across all non-redundant collection of HEV-protease domains, were derived (Fig. 1B).

3.2. Very close structural homology of HEV-protease with RUBV-p150

The PRFsec-predicted secondary structure revealed elemental composition: H (15.62%), E (33.75%) and L (50.62%) in HEV-protease (data not shown). For model building, the HEV consensus target (a.a. 434–563) when aligned with RUBV-p150 and FMDV-Lpro sequences, showed the HEV-protease essential cysteines and histidines in a close homology with RUBV-p150 (Fig. 2A). Interestingly, the PSIPRED-prediction of the secondary structure of HEV-protease was in a very close structural-homology with that of RUBV-p150 (Fig. 2B).

3.3. A 3D model of HEV-protease

The consensus-prediction results of RUBV-p150 structural template allowed us, for the very first time, to construct a 3D model of HEV-protease with active ‘cysteine-histidine’ backbone. Of the five initial models constructed, the one with lowest DOPE (data not shown) was selected for further analysis. In the absence of a crystal-structure, our predicted model of HEV-protease was very close to experimentally and structurally characterized RUBV-p150 (Fig. 3). Like p150, the predicted ‘papain-like β-barrel fold’ in our model structurally confirmed the linear sequence homology-based classification of HEV-protease (Koonin et al., 1992).

3.4. Recognition of a putative ‘catalytic dyad’ in the ‘papain-fold’

Our comparative analysis of the viral protease models recognized the HEV ‘Cys434-His443’ homologue (Fig. 3) of RUBV ‘Cys1152-His1273’ that constituted the putative ‘catalytic dyad’ in the ‘papain-fold’.

3.5. Identification of a putative ‘Zn²⁺-binding motif’

Based on homology analysis, we identified a ‘Zn²⁺-binding motif’ within the framework of HEV-protease ‘papain-like fold’. Similar to RUBV-p150 ‘Cys1167-Cys1175-Cys1178-Cys1225-Cys1227’ residues, clusters of ‘Cys457-His458-Cys459’ and ‘Cys481-Cys483’ were arranged closely in the proposed model that could form a potential Zn²⁺-binding pocket (Fig. 3).

3.6. Identification of a putative ‘EF-hand Ca²⁺/CaM-binding motif’

Our further scanning for RUBV ‘EF-hand Ca²⁺/CaM-binding motif’ in HEV-protease identified partially homologous sequences.

While the catalytic ‘His1273’, was excluded in RUBV ‘Ca²⁺/CaM-binding’ sequences, both ‘Cys434’ and ‘His443’ of the dyad remained out of its HEV equivalent (Fig. 3). By homology, we therefore, proposed the ‘D-(X)⁶-[DEA]-(X)₂⁰-[R[WL]GQ]-D-(X)₁⁷-[DNEAE]-G-X-D-X-D-(X)₁⁰-G-(X)₂⁰-D-(X)₁₉-D-X-DG’ residues (a.a. 445–549) in the HEV-protease model.

3.7. Formations of ‘disulfide-bridges’ between active cysteines

A DISULFIND program-based analysis of protein secondary structure further confirmed formations of three disulfide-bridges between Cys434-Cys481, C471-Cys483 and Cys472-Cys563 residues in the HEV-protease (data not shown).
3.8. Recognition of a putative ‘WW/rsp5-domain’

Two highly conserved tryptophan (W437 and W476) residues were observed in HEV-protease domain (Fig. 1B) where W476 clustered in the predicted ‘EF-hand loop’. The W476 could pair with the upstream-located W437 to form a putative ‘WW/rsp5’ module of the proposed HEV-protease model (Fig. 3).

4. Discussion

The best characterized family of cysteine proteases is the ‘papain’ that includes mammalian lysosomal cathepsin where the ‘papain-fold’ catalytic dyad (Cys25-His159) is evolutionarily preserved in all known eukaryotic papain-like cysteine proteases (Grzonka et al., 2001). In HCoV-229E PL1pro, the conserved catalytic ‘Cys1054-His1205’ dyad was found to be indispensable for its proteolytic activity (Herold et al., 1998). Further, the RUBV-p150, Cys1152-His1273 and FMDV-Lpro, ‘Cys51-His148’ have been reported as Zn2+ dependent catalytic dyads (Liu et al., 2000; Chen et al., 1996; Gorbalenya et al., 1991). Our comparative analysis thus, recognized the HEV ‘Cys343-His443’ homologue of RUBV that constituted the putative catalytic dyad. This was however, in contrast with the previously sequenced homology-based prediction of ‘Cys343-His590’ as catalytic residues in the HEV-protease (Koonin et al., 1992). Moreover, in the Mexican and Burmese strains, ‘His590’ is substituted with ‘leucine’ and ‘tyrosine’, respectively, showing non-conservational dispensability of histidine. A previous alignment analysis of FMDV-Lpro with the equivalent regions of equine rhinitis virus (ERV-1 and ERV-2) polyproteins had shown complete conservation of the active ‘Cys51’ amongst the three aphthoviruses but not ‘His148’, except ERV-2 (Gorbalenya et al., 1991). This also supports the non-conservation and positional dispensability of the second catalytic ‘histidine’ in viral thiol-proteases, in general. Although, the conserved ‘Cys434’ and non-conserved ‘His590’ was found non-essential in HEV viability, the indispensability of highly conserved ‘His443’ (Parvez, 2013) does support our structural recognition of the catalytic dyad. Very reasonably, such a structural diversity of cysteine proteases could be related to the extremely high mutation rate of the viral RNA genomes. As a result, many viral proteases have functionally diverged to the point where sequence similarity between homologues can be rarely detected.

Several RNA virus papain-like proteases, such as the PL2 of SARS-coronavirus (SARS-CoV) (Han et al., 2005), nsp1 of equine arteritis virus (EAV) (Tijms et al., 2001), the Lpro of encephalomyocarditis virus (EMV) (Dvorak et al., 2001), the NS3 of HCV (Barbatto et al., 1999), the PL1pro of HCoV-229E, the p150 of RUBV (Zhou et al., 2007) and Vpr of FMDV (Guarne, 2001) are shown to have ‘Zn2+ binding motifs’. We therefore, using the RUBV-p150 as molecular and structural surrogate-model, identified a ‘Zn2+ binding motif’ homologue of HEV-protease. Structural Zn2+-binding sites are generally coordinated by four cysteines and a histidine as ligands (Zhou et al., 2007). In line with this, a ‘His458’ was also clustered with four cysteines in our predicted Zn2+-binding pocket, unlike RUBV-p150 model where no histidine was present among five cysteines (Fig. 3). However, the human rhinovirus 2 (HRV2) and poivovirus 2A proteases Zn-binding motifs, ‘Cys52-Cys54-Cys112-His114’ (Sommergruber et al., 1997) and ‘Cys55-Cys57-Cys115-His117’ (Yu and Lloyd, 1992), respectively, has three cysteines plus a histidine. As understood, such structural and functional diversity could be clearly attributed to the extreme mutability and host-adaptability of RNA viruses in their evolutionary processes. Notably, the critical role of Zn2+-binding papain-like proteases in viral life cycles are well known. Previously, using the genomic replicon-system, the RUBV-p150, ‘Cys1175, Cys1178, Cys1225 and Cys1227’ residues are shown essential in its replication (Zhou et al., 2007). In line with this, our recent analysis of the HEV-protease domain nonviable mutants of Cys457, Cys459, Cys481 and Cys483 residues (Parvez, 2013), strongly support potentiality of the predicted ‘Zn2+ binding motif’.

Fig. 3. Proposed 3D-structural model of HEV-protease, showing a ‘papain-like β-barrel’ fold (green) and the structural positions of active cysteines (red) and histidines (blue) that constitute the putative ‘catalytic dyad’, ‘Zn2+ binding motif’ and ‘Ca2+ binding EF-hand’. Compare with the well characterized model of RUBV-p150 (inset; adopted and modified from Zhou et al., 2007) for the HEV-protease structural homology. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
Recently, a Ca$^{2+}$-dependent association of calmodulin (CaM) with RUBV-p150 has led to map a unique ‘EF-hand Ca$^{2+}$/CaM-binding motif’ (CWLRAAANVAQARAGYTSAGCPKCAYG; a.a. 1152–1182) that was required for its optimal stability under physiological conditions and virion infectivity (Zhou et al., 2007). In the RUBV-p150 model, the ‘Ca$^{2+}$/CaM-binding motif’ partially overlaps the ‘Zn$^{2+}$-binding motif’, including the catalytic ‘Cys1152-His1273’ dyad (Zhou et al., 2007). In our predicted model, while the catalytic ‘H1273’, was excluded in RUBV Ca$^{2+}$/CaM’ binding sequences, both ‘Cys434’ and ‘His443’ of the dyad remained out of its HEV equivalent. Moreover, the canonical ‘EF-hand motif’ signature ‘D-X-[DNS]-[ILVFYW]-[DEN]-G-[GP]-XX-DE’ is described to form the Ca$^{2+}$-binding loops in living systems (Zhou et al., 2007). Notably, presence of the essential cysteine residues in the overlapping putative ‘Ca$^{2+}$/CaM-binding motif’, also suggested formation of intramolecular disulfide-bridges between cysteines that might structurally facilitate the orientation of the ‘EF-hand’ towards Ca$^{2+}$ binding. Our analysis of protein secondary structure also confirmed formations of three disulfide-bridges between active cysteines.

The ‘WW-domain’ or ‘rps5-domain’ formed by two distantly located tryptophans (W; >20 residues apart, in general) is known as one of the smallest protein modules that specifically interacts with ‘proline-rich motif’ of regulatory proteins (Sudol et al., 1995). This is however, not observed in proteins, including nonstructural cysteine proteases so far. In our proposed model, W437 is therefore predicted to pair with the upstream-located W437 that could form the putative ‘W437-W476/rs5-domain’. Like closely-related virus polyproteins, HEV ORF1 also contains a ‘proline-rich hypervariable region’ followed by the protease-domain (Fig. 1A) that indicates its possible interaction with the viral protease through predicted ‘W437–W476’ module.

In conclusion, our present model of HEV-protease supported by molecular data, warrants critical roles of the putative ‘catalytic dyad’ and ‘divalent metal-binding motifs’ in viral protease structural-integrity, ORF1 self-processing, and RNA replication. These results are however, not a confirmation of the structures presented, but do present testable hypotheses about the structure and function of the HEV-protease. Therefore, experimental validations involving molecular, biochemical and biophysical methods are further needed. Knowledge of the structure of HEV-protease would be thus, valuable for understanding the virus biology and classification as well as antiviral drug development.

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References

Ansari, I.H., Nanda, S.K., Durgapal, H., et al., 2000. Cloning, sequencing, and expression of the hepatitis E virus (HEV) nonstructural open reading frame 1 (ORF1). J. Med. Virol. 60, 275–283.

Barbato, C., Cicero, D.O., Nardi, M.C., et al., 1999. The solution structure of the N-terminal protease domain of the hepatitis C virus (HCV) NS3 protein provides new insights into its activation and catalytic mechanism. J. Mol. Biol. 289, 371–384.

Chen, Y., Carrington-Lawrence, S.D., Bai, P., Weller, S.K., 2005. Mutations in the putative zinc-binding motif of US52 demonstrate a complex interdependence between the US5 and US52 subunits of the human herpes simplex virus type 1 helicase/primase complex. J. Virol. 79, 9088–9096.

Chen, J.P., Strauss, J.H., Strauss, E.G., Frey, T.K., 1996. Characterization of the rubella virus nonstructural protease domain and its cleavage site. J. Virol. 70, 4707–4713.

Dvorak, C.M., Hall, D.J., Hill, et al., 2001. Leader protein of encephalomyocarditis virus binds zinc, is phosphorylated during viral infection, and affects the efficiency of genome translation. Virology 290, 261–271.

Fukuoka, N., Huang, C., Kiyotani, et al., 2002. Mutational analysis of the Sendai virus V protein: importance of the conserved residues for Zn binding, virus pathogenesis, and efficient RNA editing. Virology 299, 172–181.

Gorbunova, A.E., Koonin, E.V., Lai, M.M., 1991. Putative papain-related thiol proteases of positive-strand RNA viruses, identification of rubi- and aphthovirus proteases and delineation of a novel conserved domain associated with proteases of RNA, alpha- and coronaviruses. FEBS Lett. 288, 201–205.

Gorelick, R.J., Chabot, D.J., Ott, D.E., et al., 1996. Genetic analysis of the zinc finger in the Moloney murine leukemia virus nucleocapsid domain: replacement of zinc-coordinating residues with other zinc-coordinating residues yields non- infectious particles containing the viral RNA. J. Virol. 70, 910–918.

Grzonka, Z., Jankowska, E., Kasprzykowski, et al., 2001. Structural studies of cysteine proteases and their inhibitors. Acta Biochim. Pol. 48, 1–20.

Guarne, A., Hampolzel, B., Glaser, et al., 2000. Structural and biochemical features distinguish the foot-and-mouth disease virus leader protease from other papain-like enzymes. J. Mol. Biol. 302, 1227–1240.

Han, Y.S., Chang, G.G., Joo, C.G., et al., 2005. Papain-like protease 2 (PLP2) from severe acute respiratory syndrome coronavirus (SARS-CoV): expression, purification, characterization, and inhibition. Biochemistry 44, 10345–10359.

Herdin, J., Gorbunova, A.E., Thié, V., Schelle, B., Siddell, S.G., 1998. Proteolytic processing at the amino terminus of human coronavirus 229E gene 1-encoded polyproteins: identification of a papain-like protease and its substrate. J. Virol. 72, 910–918.

Holla, R.P., Ahmad, I., Ahmad, Z., Jameel, S., 2013. Molecular virology of hepatitis E virus. Semin. Liver Dis. 33, 3–14.

Kamar, N., Izopet, J., Rostaing, L., 2013. Hepatitis E virus infection. Curr. Opin. Gastroenterol. 29, 271–278.

Karlo, O.A., Koles, K., 2011. Deubiquitination activity associated with heparin virus ORF3 papain-like cysteine protease. J. Gen. Virol. 92, 2088–2092.

Liu, X., Yang, J., Ghazi, A.M., Frey, T.K., 2000. Characterization of the zinc binding activity of the rubella virus nonstructural protease. J. Virol. 74, 5949–5956.

Mark-Danieli, M., Laham, N., Kenan-Eichel, et al., 2005. Single point mutations in the zinc finger motifs of the human immunodeficiency virus type 1 nucleocapsid alter RNA binding specificities of the gag protein and enhance packaging and infectivity. J. Virol. 79, 7756–7767.

Parvez, M.K., 2013. Molecular characterization of hepatitis E virus ORF1 gene supports a papain-like cysteine protease (PCP)-domain activity. Virus Res. 178, 553–556.

Perttilä, J., Spaul, P., Ahola, T., 2013. Early secretory pathway localization and lack of processing for hepatitis E virus replication protein pORF1. J. Gen. Virol. 94, 807–816.

Ropp, S.L., Tam, A.W., Beames, B., Purdy, M., Frey, T.K., 2000. Expression of the hepatitis E virus ORF1. Arch. Virol. 145, 1321–1337.

Rost, B., Yachdav, G., Liu, J., 2004. The PredictPCP. server. Nucleic Acids Res. 32, 321–326.

Ryan, M.D., Flint, M., 1997. Virus-encoded proteinases of the picornavirus supergroup. J. Gen. Virol. 78, 699–723.

Sehgal, D., Thomas, S., Chakraborthy, M., Jameel, S., 2006. Expression and processing of the Hepatitis E virus ORF1 nonstructural polyprotein. J. Virol. 79, 229–236.

Sommergruber, W., Seipel, J., Fessl, F., Skern, T., Liebig, H.D., Casari, G., 1997. Mutational analyses support a model for the HRV2 2A protease. Virology 234, 203–214.

Sudol, M., Chen, H.J., Bougeret, C., Einbond, A., Bork, P., 1995. Characterization of a novel protein-binding module—the WW domain. FEBS Lett. 369, 67–71.

Tellingshusien, T.L., Marcotrigiano, J., Gorbunova, A.E., Rice, C.M., 2004. The N53A protein of hepatitis C virus is a zinc metalloprotein. J. Biol. Chem. 279, 48576–48587.

Tjima, M.A., van Dinten, L.C., Gorbunova, A.E., Snijder, E.J., 2001. A zinc-finger-containing papain-like protease couples subgenomic mRNA synthesis to genome translation in a positive-stranded RNA virus. Proc. Natl. Acad. Sci. U. S. A. 98, 1889–1894.

Yu, S.F., Lloyd, R.E., 1992. Characterization of the roles of conserved cysteine and histidine residues in poliovirus 2A protease. Virology 186, 725–735.

Zhou, Y., Tseng, W.P., Yang, W., et al., 2007. Identification of a Ca$^{2+}$/CaM-binding domain in the rubella virus nonstructural protein. J. Virol. 81, 7517–7528.

Zhou, Y., Tseng, W.P., Ye, Y., et al., 2009. A cysteine-rich metal-binding domain from rubella virus non-structural protein is essential for viral protease activity and virus replication. Biochem. J. 417, 477–483.