Viral Macrodomains: Unique Mediators of Viral Replication and Pathogenesis

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Keywords: macrodomain, ADP-ribosylation, Poly-ADP-ribose polymerase (PARP), interferon (IFN), replication, pathogenesis, Coronaviridae, Togaviridae, Hepeviridae
Abstract
Viruses from the Coronaviridae, Togaviridae, and Hepeviridae families all encode genes that contain a conserved protein domain, called a macrodomain; however, the role of this domain during infection has remained enigmatic. The recent discovery that mammalian macrodomain proteins enzymatically remove ADP-ribose, a common post-translation modification, from proteins has led to an outburst of studies describing both the enzymatic activity and function of viral macrodomains. These new studies have defined these domains as de-ADP-ribosylating enzymes, which indicates that these viruses have evolved to counteract antiviral ADP-ribosylation, likely mediated by poly-ADP-ribose polymerases (PARPs). Here, we will comprehensively review this rapidly expanding field, describing the structures and enzymatic activities of viral macrodomains, and discussing their roles in viral replication and pathogenesis.
ADP-ribosylation

ADP-ribosylation is a posttranslational modification (PTM) that was identified serendipitously over 50 years ago. While studying RNA synthesis, Chambon and colleagues discovered a large increase in $^{32}$P incorporation when adding nicotinamide mononucleotide (NMN), a precursor of NAD⁺, into liver nuclear extracts in the absence of other nucleotides [1]. The product was determined to be polymers of ADP-ribose, opening-up a whole new field of investigation.

ADP-ribosylation is a ubiquitous modification present across all domains of life [2]. ADP-ribose is added to proteins by ADP-ribosyl transferases (ARTs) – a large family of homologous proteins with similarity to diphtheria and cholera toxins [3]. Based on these similarities ARTs can be classified as ARTDs (diphtheria toxin-like) and ARTCs (cholera toxin-like), respectively [4]. These enzymes use nicotinamide adenine dinucleotide (NAD⁺) as a substrate to transfer ADP-ribose onto proteins. The best understood group of ARTs are poly-ADP-ribose polymerases (PARPs), which in humans consists of 17 members. Modification by PARPs occurs largely on acidic residues (glutamate and aspartate) but serines, lysines, arginines, and cysteines can also act as acceptors [2]. PARPs are the only ART group that can synthesize poly-ADP-ribose (PAR) on proteins (chains of repeating ADP-ribose units), but only some PARPs have PARylating activity (PARPs 1/2/5a/5b). Thus, most PARPs are only known to transfer a single ADP-ribose to their target protein [5].

ADP-ribosylation has a large array of effects on proteins, including modification of enzymatic activity, promoting the ubiquitination and degradation of proteins, and acting as a platform for protein interactions. Several protein domains are known to bind to ADP-ribose, including the WWE, PAR-binding motif (PBM), PAR-binding zinc finger (PBZ), and
macrodomains [6]. ADP-ribosylation is well-known for its role in the DNA damage response, mediated by PARP1-dependent ADP-ribosylation of histones and other proteins which recruits repair proteins to sites of DNA damage [7]. In addition, the Tankyrases (PARP5a/5b) control Wnt signaling [8]. Recently, more defined roles for the MARylating PARPs have been described, including a prominent role in the cellular stress response. PARP16 regulates the ER-stress response [9], and several PARPs are present in cytoplasmic stress granules (PARPs 12,13,14,15, and 5a) [10]. ADP-ribosylation of Argonaute proteins in stress granules by an unknown PARP inhibits RNA interference [10, 11]. Finally, PARPs have both anti- and pro-viral activities (Box 1).

As with any PTM, ADP-ribose must be removed in a timely manner to prevent an untoward response and to recycle ADP-ribose components (Figure 1). The cellular enzymes that remove PAR from proteins are PAR glycohydrolase (PARG), and to a lesser extent ADP-ribosyl hydrolase [12, 13]. The importance of removing PAR is demonstrated by the embryonic lethality of mice with genetic deletion of PARG [14]. However, PARG only cleaves between the unique glycosidic bonds of PAR [15], and thus it was unclear how the terminal ADP-ribose moiety was removed from the modified protein. This conundrum was resolved by the discovery that cellular macrodomain-containing proteins, including terminal ADP-ribose glycohydrolase 1 (TARG1), MacroD1, and MacroD2 removed this terminal ADP-ribose [16-18]. The human genome contains at least 11 macrodomain containing genes, but the substrate specificity and physiological roles of these proteins are largely unknown.

Almost 3 decades ago, bioinformatics approaches identified a unique conserved gene domain within three viral families, the Togaviridae, Coronaviridae, and Hepeviridae [19-21]. All of these families are positive-sense RNA viruses and cause disease in humans and animals (Box
2). The domain was named the “X” domain as its structure and function was unknown. These domains were defined as “macrodomains” after crystal structures of both cellular and viral macrodomains revealed a core fold homologous to the non-histone part of the macroH2A protein [22-24]. These macrodomains were shown to have phosphatase activity and were named ADP-ribose-1”-phosphatases (ADRPs) [23, 25-28].

Despite the demonstrated ADRP activity, it was unclear why viruses would devote a gene product to converting ADP-ribose-1”-phosphate to ADP-ribose. The discovery that macrodomains de-ADP-ribosylate proteins strongly suggested that their major role was in reversing anti-viral ADP-ribosylation. However, this hypothesis has yet to be proven. As all 3 viral families that contain macrodomains include established human pathogens, understanding how macrodomains contribute to replication and pathogenesis is important. Here we will comprehensively review the structures and enzymatic activities of these viral macrodomains, describe their potential functions and discuss future challenges in understanding how macrodomains impact virus biology.

Viral Macrodomain Structure and Enzymatic Activity

Macromodules are conserved, ~150 amino acid protein domains present in all kingdoms of life. Based on phylogeny, macrodomains are divided into different classes. Most RNA virus macromodules fall into the MacroD-like family, which include human macrodomains MacroD1 and MacroD2 [29]. The exceptions are the SARS Coronavirus (SARS-CoV) unique macrodomains (Mac2/Mac3 – formerly known as SUD domains), which we will not further discuss as they are distinct from the conserved macrodomain (Mac1).
Macrodomes adopt a globular, mixed $\alpha/\beta/\alpha$ sandwich fold, first shown when the structure of the archaeal macrodomain AF1521 [22] was determined. Many viral macrodomain structures, including those of Alphaviruses and Coronaviruses (CoVs), but not from Hepatitis E virus, have been determined, with 23 PDB entries available (www.rcsb.org). The globular macrodomain contains a conserved cleft that has been shown to bind ADP-ribose [30], a feature that has been confirmed for viral macrodomains by numerous biochemical studies [27, 31-33]. Some of the most conserved residues of macrodomains are located at the surface and near the ADP-ribose binding cleft (Figure 2).

Based on ADP-ribose-macrodomain complex structures, these conserved residues provide high specificity and affinity for ADP-ribose. The distal ribose moiety is especially tightly coordinated by key residues found in loop 1 (between $\beta$-sheet 2 and $\alpha$-helix 2) and loop 2 (between $\beta$-sheet 5 and $\alpha$-helix 5) (Figure 2). Loop 1 contributes backbone contacts to the ADP-ribose $\alpha$-phosphate and 1” and 2” OH groups of the distal ribose. In addition, the conserved asparagine residue (N41 in SARS-CoV) makes a hydrogen bond to the 2” OH group. In CoV macrodomains, the conserved histidine residue (H46) contributes to ADP-ribose binding through a coordinated water. Alphavirus macrodomains have a conserved cysteine (C34) that could fulfil a similar role, whereas human MacroD2 contains an aspartic acid (D102) that makes a hydrogen bond with the 2” OH group. Loop 2 contributes backbone contacts primarily to the $\beta$-phosphate of ADP-ribose. In addition, isoleucine (I132) and phenylalanine (F133) residues provide van der Waals contacts and are thought to direct orientation of the distal ribose [16, 24, 27, 34].

In some macrodomains, tyrosine replaces phenylalanine and forms a hydrogen bond to the 3” OH of ADP-ribose. Macrodomes with a threonine before the G131 residue contribute an additional hydrogen bond to the 3’ OH of the proximal ribose [e.g. T111 in Chikungunya].
virus (CHIKV)]. Besides the two loops, other notable ADP-ribose binding residues are D23
[N10 in Sindbis virus (SINV) and S28 in hepatitis E virus (HEV)], which contacts the amino
group of adenine, and N157 (R144 in CHIKV and F224 in MacroD2), which interacts with the
adenine ring via $\pi$-charge or $\pi-\pi$ interactions. Depending on the ADP-ribose contacting residues,
the affinities for ADP-ribose by different macrodomains vary greatly with dissociation constants
ranging from 0.1 to above 20 $\mu$M [16, 27, 31, 32].

**Viral Macrodomain Enzymatic Activity**

Binding of ADP-ribose is not the sole property of macrodomains. The first enzymatic
activity ascribed to macro-D macrodomains was the dephosphorylation of ADP-ribose-1''-
phosphate (a by-product of tRNA splicing reactions) identified through a biochemical-genomic
screen in yeast [25]. This phosphatase activity was also detected for viral macrodomains [24, 27,
28, 31, 32]. However, the enzymatic activity of macrodomains is low, the activities vary across
different virus species, and no easy explanation exists as to why ADP-ribose-1''-phosphate
hydrolysis would benefit these viruses. Therefore, other functions of viral macrodomains were
considered. They include but are not limited to: binding ADP-ribosylated proteins, poly-ADP-
ribose, RNA or other nucleic acids [31].

The discovery that macroD-like macrodomains are de-ADP-ribosylating enzymes [16, 17]
suggested that viral macrodomains could have the same activity. Indeed, Li *et al.* were the first to
show both de-MAR-ylating and de-PAR-ylating activities for macrodomains from members of
all three macrodomain-containing virus families [35]. Fehr *et al.* confirmed and additionally
tested the effects of several mutations on the SARS-CoV macrodomain de-ADP-ribosylating
activity [36]. Eckei *et al.* demonstrated de-ADP-ribosylating activity of macrodomains from
different Alphaviruses, addressing both de-MAR-ylating and de-PAR-ylating activities [37],
however, it is still unknown which features of the macrodomain limit its activity to de-MAR-ylation [16] and which are required for de-PAR-ylation [35, 37]. Finally, McPherson et al. showed de-MAR-ylating activity for the Chikungunya virus macrodomain and determined that this macrodomain removes ADP-ribose from acidic residues but not from lysines [38]. Together these four studies strongly suggest that de-ADP-ribosylation is likely the biochemical function of viral macrodomains. ADP-ribosylated proteins are linked to ADP-ribose through the 1'' position, reminiscent of ADP-ribose-1''-phosphate, explaining how macrodomains could have both activities. These latest studies have shifted the focus to de-ADP-ribosylation, but other potential macrodomain functions should not be neglected, as the catalytic mechanism of de-ADP-ribosylating macrodomains is not fully understood and the residues impacting de-ADP-ribosylation often affect ADP-ribose binding and other catalytic activities (e.g. ADP-ribose-1''-phosphate hydrolysis).

Macrodomain mutations experimentally shown to disrupt catalytic function are summarized in Table 1. The corresponding residues in different viral macrodomains are identified by sequence alignment and their relation to the ADP-ribose substrate are visualized in Figure 2. The mutational approaches interfere with macrodomain function by i) reducing ADP-ribose binding affinity (e.g. D23A (SARS-CoV), T111A (CHIKV), Y114F (CHIKV)), ii) introducing steric hindrance (e.g. G131V (SARS-CoV), G123A (HEV), G112E (CHIKV)), or iii) displacing potential catalytic water and reducing conformational strain on the distal ribose (e.g. G32E+V113R+Y114N (CHIKV)).

The most common studied mutation is substitution of the highly-conserved asparagine to alanine (N41A in SARS-CoV). This asparagine coordinates the 2'' OH of distal ribose and was proposed to be essential for catalysis. While the N41A mutation abolishes the catalytic activity
of SARS-CoV macrodomain [27, 28, 36], it reduces but does not abolish the catalytic activity of
other cellular and viral macrodomains [16, 37, 38]. Such apparent discrepancy could be
explained by reduced ADP-ribose binding by the asparagine-to-alanine mutant since viral
macrodomains, especially the CoVs, have a lower affinity for ADP-ribose (20 µM in SARS-CoV
compared to 0.15 µM in human MacroD2). The loss of a single hydrogen bond between the CoV
macrodomain and ADP-ribose could reduce its affinity to a point where the macrodomain
enzymatic activity is non-detectable. An alternative explanation is that different catalytic
mechanisms exist among macro-D type macrodomains. Despite these uncertainties, the strict
conservation of this asparagine and the effects of its mutation on in vitro activity and virus
fitness make it a valuable tool to study viral macrodomains.

The fact that viral macrodomains are generally required for virulence (see section below)
suggests that small molecule inhibition of macrodomains could be a novel therapeutic approach
to preventing severe Alphavirus, Hepatitis E virus, or Coronavirus-induced disease. One concern
in considering the development of such molecules is the potential difficulty in specifically
targeting the viral macrodomains but not the cellular MacroD1 or MacroD2 proteins. While such
concern is warranted, there is potential for exploiting differences between human and viral
macrodomains. Residues contributing to distal ribose 2’’ OH coordination differ between human
and viral macrodomains. In addition, the space around the proximal ribose and the surface
residues contacting the adenine-ring might be additional areas of interest for specific small
molecule design. The potential for virus escape by mutating at these differing residues could
hamper the feasibility of inhibitor design and usability, but these concerns could be addressed
experimentally. The observation that even a 50% reduction in viral macrodomain activity greatly
reduced virus fitness suggests that viruses would struggle to escape potential inhibitors [38]. If
the risk of off-target effects in humans is too high, such inhibitors could be used in veterinary medicine.

Role of Viral Macrodomains During Infection

In recent years, significant progress has been made in deciphering the role of viral macrodomains, largely due to the advent of reverse genetic methods for these viruses. In this section, we will review the known and potential roles for viral macrodomains.

Hepatitis E Virus Macrodomain

HEV is a non-enveloped positive-sense RNA virus with a genome of 7.2 kb that contains 3 open reading frames: ORF1, ORF2, and ORF3. The HEV macrodomain is a part of the multi-domain ORF1 gene product of HEV (Figure 3) [39]. In a study on the ORF1 gene products of HEV, Nan et al. found that ORF1 inhibited poly I:C-dependent induction of interferon-β (IFN-β) [39]. Using overexpressed proteins, only the papain-like cysteine protease and the macrodomain could block IFN-β expression. Importantly, the HEV macrodomain inhibited both IKKe overexpression-induced IFN-β expression and interferon regulatory factor-3 (IRF-3) phosphorylation. Since the protein kinase IKKe phosphorylates IRF-3, these data suggest that the macrodomain targets IRF-3. However, macrodomain inhibition of IKKe-induced IFN-β expression was significantly less than its inhibition of poly I:C-induced IFN-β expression, suggesting other mechanisms are involved. In a study by Ojha and Lole, the HEV macrodomain was found to interact with the light chain subunit of human ferritin (FTL), which led to a reduction of secreted ferritin [40]. Ferritin is an iron binding protein that helps the cell store free iron. Ferritin complexed with iron can activate NF-κB-dependent IFN production, suggesting this interaction could be a mechanism of innate immune suppression [41]. Neither of these
studies addressed whether the ability of the HEV macrodomain to bind or hydrolyze ADP-ribose was important for these observations.

Utilizing full-length replicon-based reverse genetic systems with either a GFP or luciferase reporter, Parvez et al. and Li et al. tested the role of the macrodomain in HEV replication [35, 42]. In these studies, several amino acids in the active site were mutated (N806, N809, H812, G815, G816, G817) and tested for their ability to replicate. In the Parvez study, all mutations produced wild-type levels of viral RNA, but only N806 and G815 mutants were viable as measured by GFP expression. Li et al. found that replication strongly correlated with the enzymatic activity of each mutation they created. These results demonstrate that the HEV macrodomain is important for replication and acts after RNA replication, likely prior to translation of viral proteins. Another study found that the HEV macrodomain interacted with the viral methyltransferase and ORF3 using yeast-2-hybrid and co-immunoprecipitation methods [43]. It will be of interest to determine if these proteins are ADP-ribosylated and whether these interactions are important for replication.

**Togavirus Macrodomain**

Togaviruses are positive-sense enveloped viruses with 10-12 kb RNA genomes. The first 2/3 of the genome encodes for 4 non-structural proteins while the final 1/3 of the genome encodes for structural genes. The macrodomain is located at the N-terminus of nsP3 (Figure 3) [44]. In 2009 Park and Griffin published a study focusing on two asparagine mutants (N10A and N24A) in the ADP-ribose binding pocket of the SINV macrodomain [44]. These mutant viruses replicated normally in BHK-21 cells but were modestly attenuated in neuronal cells and significantly attenuated in 2-week-old mice. Upon replication in neurons the N10A mutation changed to threonine or aspartic acid, and a second site mutation of a glutamic acid at position 31...
to a glycine appeared. Most macrodomains have a glycine at this position, suggesting this
mutation may enhance macrodomain activity. It was unclear how these mutations affected this
protein, as neither mutant affected PAR binding and it was unknown whether alphaviruses’
macrodomains had de-ADP-ribosylating activity. However, recently it was demonstrated that
CHIKV contains these activities [37, 38], making it likely that these mutations within the ADP-
ribose binding pocket of the SINV macrodomain attenuated its enzymatic activity.

As opposed to SINV, mutations in the active site of the CHIKV macrodomain had severe
effects on virus replication [38]. Three recombinant viruses with mutations that severely affected
both ADP-ribose binding and hydrolase activity were unrecoverable as they quickly reverted to
wild-type (D10A, G32E, G112E). The G32E mutation even reverted when transfected into an
*Aedes albopictus* cell line, suggesting that anti-viral ADP-ribosylation also occurred in mosquito
cells. Other mutations that partially ablated hydrolase activity and ADP-ribose binding (T111A,
G32S, G32A) replicated at 1 to 2 logs lower efficiency than wild-type virus in a neuronal cell
line (NSC-34). Finally, one mutation, Y114A, was identified that had enhanced ADP-ribose
binding but decreased hydrolase activity. This mutation was also significantly attenuated in
NSC-34 cells, demonstrating that ADP-ribosyl hydrolase activity alone is important for
replication. In this study, de-ADP-ribosylating activity, replication, and virulence did not
perfectly correlate, suggesting a role for ADP-ribose binding in virulence. Of note, an additional
mutant, R144A, which had normal levels of ADP-ribose binding and hydrolase activity *in vitro*,
quickly reverted in tissue culture cells. This mutation could affect alternative functions of the
macrodomain. For example, studies have found that mutations in the ADP-ribose binding
domain of the VEEV macrodomain could compensate for mutations in other regions of the
genome, however it is unclear whether these activities were related to de-ADP-ribosylating
activity or other functions [45-47]. Additionally, Lulla et al. found that C-terminal amino acids of the Semliki Forest Virus (SFV) macrodomain are required in the processing of the nsP2/3 cleavage site, which could explain the defect of the R144A mutation [48].

**Coronavirus Macrodomain**

CoVs are large, positive-sense enveloped RNA viruses with ~30kb genomes. The N-terminal two-thirds of the CoV genome contains 16 non-structural proteins (NSPs) while the C-terminal one-third of the genome contains the structural and accessory genes. The conserved macrodomain is located at the N-terminus of the multi-domain nsp3 protein (Figure 3) [49]. In SARS-CoV and possibly other CoVs, two additional macrodomains (Mac2/Mac3) immediately follow the conserved macrodomain (Mac1) [50]. Mac2 and Mac3 do not bind or hydrolyze ADP-ribose; in contrast, they bind G quadruplexes [51]. Using a SARS-CoV replicon system, Kusov et al. found that Mac3 was essential for replication [52].

Most studies of the conserved CoV macrodomain (Mac1) function have used recombinant viruses with the highly-conserved asparagine mutated to an alanine. This mutation has small to no effects on CoV replication in cell culture [28, 36, 49, 53, 54]. Also, deletion of the macrodomain had no effect on replication of a SARS-CoV replicon [52].

In marked contrast, several studies have found that the CoV macrodomain is essential for viral pathogenesis. Eriksson et al. first showed that the macrodomain was required for murine hepatitis virus (MHV) strain A59-induced hepatitis [53] while Fehr et al. demonstrated that mutation of the macrodomain of an encephalitic strain of MHV and of mouse-adapted (MA) SARS-CoV resulted in attenuation *in vivo* [36, 49]. In all cases, the mutation was associated with reductions in viral load. It was also shown by both Eriksson et al. and Kuri et al. that the macrodomain could repress IFN production; however, it was unclear if this occurred *in vivo* and
whether it was important for pathogenesis [53, 54]. Fehr et al. found that the SARS-CoV
macrodomain repressed IFN and cytokine production both in mice and in a bronchial epithelial
cell line [36]. A co-infection with wild-type and the macrodomain mutant SARS-CoV
demonstrated that the enhanced interferon response was partially protective in mice. Also, the
reduced viral load observed in SARS-CoV-infected mice was independent of the IFN response,
as viral loads were not increased in IFNAR−/− mice infected with macrodomain mutant virus. In
combination, these studies led to the conclusion that the SARS-CoV macrodomain promotes
replication in vivo and independently suppresses the IFN response, with both functions playing a
role in pathogenesis.

**Concluding Remarks**

Despite the discovery that the viral “X” domains had highly conserved structures with
cellular macrodomains ~15 years ago, the function of these domains remained an enigma until
recent discoveries showed that viral macrodomains remove ADP-ribose from proteins. While
this activity has not been demonstrated during infection, the publications discussed in this review
strongly suggest that this is the case. As such, the primary focus of macrodomain research will
likely shift from basic functional and biochemical studies, to identifying antiviral PARPs and the
protein targets of viral macrodomains (see Outstanding Questions). While identifying ADP-
ribosylated proteins has proven difficult in the past, new methods for detecting ADP-ribosylation
should lead to important discoveries in this field [55]. These studies will significantly enhance
our understanding of these proteins, which could lead to new approaches for treating and
preventing disease caused by macrodomain-expressing pathogens.
Acknowledgments

This research is supported by a Wellcome Trust grant (101794), and a Cancer Research UK grant (C35050/A22284) to Ivan Ahel, an NRSA award to Anthony R. Fehr (F32-113973), and National Institute of Health grants to Stanley Perlman (R01 AI129269; R01 NS365092; P01 AI060699).
Box 1. The Interplay between PARPs and Virus Infections

Several studies have found PARPs to have both pro-viral and anti-viral activities. Using PARP inhibitors, several DNA viruses, such as herpesviruses, poxviruses, and adenoviruses, have been found to require ADP-ribosylation for optimal replication [56-58]. This is not surprising given the importance of PARPs in cellular DNA replication and repair. The HSV-1 ICP0 protein enhances PARylation by degrading PAR-glycohydrolase, which removes PAR from proteins [56]. Furthermore, TiPARP was shown to ADP-ribosylate TBK-1 and decrease IFN-β production, leading to enhanced replication of several RNA and DNA viruses [59]. The best known antiviral PARP, PARP13, also known as ZAP (zinc-antiviral protein) restricts the replication of several RNA viruses by targeting viral or host RNA for degradation [60]. PARP13 contains mutations in its PARP domain making it enzymatically inactive, indicating that its antiviral activity does not require ADP-ribosylation [61]. However, PARP13 is known to be important for stress granule ADP-ribosylation, and it also contributes to the ADP-ribosylation of the overexpressed PA and PB2 proteins of Influenza A virus which leads to their ubiquitination and proteasomal degradation [10, 11, 62]. Thus, while it does not directly ADP-ribosylate proteins, it appears that PARP13 participates in this process, possibly by binding to active PARPs and recruiting them to target proteins. Similarly, PARPs 7, 10, and 12 directly inhibit alphavirus replication, but in a PARP-independent manner, as catalytically inactive mutants still blocked replication [63]. Overexpression of PARP12 has also been shown to inhibit the replication of several negative strand RNA viruses, including Vesicular Stomatitis Virus (VSV) [64]. PARP1 represses the transcription of integrated retroviruses in a chicken lymphoblastoid cell line, again in a PARP-independent manner [65]. In addition, multiple PARPs are interferon-stimulated genes (ISGs), and in silico analyses indicate that PARPs 4, 9, 14, and 15 are rapidly...
evolving, a hallmark of antiviral defense proteins [66]. However, despite these data, anti-viral
ADP-ribosylation of cellular or viral targets have yet to be identified.

Box 2. Pathogenesis of Macrodomain Containing Viruses

Hepatitis E virus (HEV) is an emerging pathogen and is probably the most common
cause of acute viral hepatitis in the world, with as many as 3 million infections per year.
Mortality rates for acute infections are ~3% for normal adults but as high as 30% for pregnant
women in their 3rd trimester, also often resulting in stillbirths of the fetus. HEV, like HCV, can
also lead to chronic hepatitis and cirrhosis [67]. In developing countries, HEV is spread by the
fecal-oral route, while in developed countries HEV is generally acquired through animal
reservoirs, most likely pigs. Treatment for HEV infection includes either monotherapy with
ribavirin or combination therapy of pegylated IFN and ribavirin. Additionally, two vaccines have
been developed for HEV that have shown to be effective [68].

The Togaviridae are divided into two genera, the Alphaviruses and the Rubiviruses.
Rubella virus, a Rubivirus, causes a congenital syndrome characterized by multi-organ birth
defects. However, this disease has largely disappeared in the US and other developed countries
since an effective vaccine was developed. Alphaviruses, which include Chikungunya virus and
Ross River virus, are transmitted by mosquitos and can infect both vertebrate and invertebrate
species. In humans, these infections can lead to a number of different pathologies, the most
common being severe arthritis or rash in humans [69, 70]. There are no alphavirus vaccines
currently available.

Coronaviruses (CoVs) cause a wide variety of diseases in both humans and mammals.
CoVs such as porcine epidemic diarrhea virus (PEDV), bovine CoV, and infectious bronchitis
virus (IBV) cause severe disease in veterinary animals. While originally only thought to be a
cause of the common cold in humans, recent epidemics have demonstrated that CoVs can also cause severe human disease. Severe acute respiratory syndrome CoV (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV) have emerged in the last 15 years causing serious respiratory disease in humans with high mortality rates (10-40%). Both viruses originated in bats and were transmitted to humans through animal reservoirs. MERS-CoV is endemic in camels in the Middle East and will likely continue to cause lethal disease for many years [71]. There are no approved vaccines or anti-viral agents for human CoVs; however several vaccines have been licensed for CoV-mediated veterinary diseases [72].
References

1 Chambon, P., et al. (1963) Nicotinamide mononucleotide activation of new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. *Biochem. Biophys. Res. Commun.* 11, 39-43

2 Palazzo, L., et al. (2017) ADP-ribosylation: new facets of an ancient modification. *FEBS J.* 284, 2932-2946

3 Holbourn, K.P., et al. (2006) A family of killer toxins. Exploring the mechanism of ADP-ribosylating toxins. *FEBS J.* 273, 4579-4593

4 Hottiger, M.O., et al. (2010) Toward a unified nomenclature for mammalian ADP-ribosyltransferases. *Trends Biochem. Sci.* 35, 208-219

5 Vyas, S., et al. (2014) Family-wide analysis of poly(ADP-ribose) polymerase activity. *Nat Commun* 5, 4426

6 Teloni, F. and Altmeyer, M. (2016) Readers of poly(ADP-ribose): designed to be fit for purpose. *Nucleic Acids Res.* 44, 993-1006

7 Messner, S. and Hottiger, M.O. (2011) Histone ADP-ribosylation in DNA repair, replication and transcription. *Trends Cell Biol.* 21, 534-542

8 Yang, E., et al. (2016) Wnt pathway activation by ADP-ribosylation. *Nat Commun* 7, 11430

9 Jwa, M. and Chang, P. (2012) PARP16 is a tail-anchored endoplasmic reticulum protein required for the PERK- and IRE1alpha-mediated unfolded protein response. *Nat. Cell Biol.* 14, 1223-1230

10 Leung, A.K., et al. (2011) Poly(ADP-ribose) regulates stress responses and microRNA activity in the cytoplasm. *Mol. Cell* 42, 489-499

11 Seo, G.J., et al. (2013) Reciprocal inhibition between intracellular antiviral signaling and the RNAi machinery in mammalian cells. *Cell Host Microbe* 14, 435-445

12 Mashimo, M. and Moss, J. (2016) Functional Role of ADP-Ribosyl-Acceptor Hydrolase 3 in poly(ADP-Ribose) Polymerase-1 Response to Oxidative Stress. *Curr Protein Pept Sci* 17, 633-640

13 Fontana, P., et al. (2017) Serine ADP-ribosylation reversal by the hydrolase ARH3. *Elife* 6, e28533
14 Koh, D.W., et al. (2004) Failure to degrade poly(ADP-ribose) causes increased sensitivity to cytotoxicity and early embryonic lethality. *Proc Natl Acad Sci U S A* 101, 17699-17704

15 Slade, D., et al. (2011) The structure and catalytic mechanism of a poly(ADP-ribose) glycohydrolase. *Nature* 477, 616-620

16 Jankevicius, G., et al. (2013) A family of macrodomain proteins reverses cellular mono-ADP-ribosylation. *Nat. Struct. Mol. Biol.* 20, 508-514

17 Rosenthal, F., et al. (2013) Macrodomain-containing proteins are new mono-ADP-ribosylhydrolases. *Nat. Struct. Mol. Biol.* 20, 502-507

18 Sharifi, R., et al. (2013) Deficiency of terminal ADP-ribose protein glycohydrolase TARG1/C6orf130 in neurodegenerative disease. *EMBO J.* 32, 1225-1237

19 Dominguez, G., et al. (1990) Sequence of the genome RNA of rubella virus: evidence for genetic rearrangement during togavirus evolution. *Virology* 177, 225-238

20 Gorbalenya, A.E., et al. (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 rubi-, alpha- and coronaviruses. *FEBS Lett.* 288, 201-205

21 Koonin, E.V., et al. (1992) Computer-assisted assignment of functional domains in the nonstructural polyprotein of hepatitis E virus: delineation of an additional group of positive-strand RNA plant and animal viruses. *Proc Natl Acad Sci* 89, 8259-8263

22 Allen, M.D., et al. (2003) The crystal structure of AF1521 a protein from Archaeoglobus fulgidus with homology to the non-histone domain of macroH2A. *J. Mol. Biol.* 330, 503-511

23 Kumaran, D., et al. (2005) Structure and mechanism of ADP-ribose-1"-monophosphatase (Appr-1"-pase), a ubiquitous cellular processing enzyme. *Protein Sci.* 14, 719-726

24 Saikatendu, K.S., et al. (2005) Structural Basis of Severe Acute Respiratory Syndrome Coronavirus ADP-Ribose-1"-Phosphate Dephosphorylation by a Conserved Domain of nsP3. *Structure* 13, 1665-1675

25 Martzen, M.R., et al. (1999) A biochemical genomics approach for identifying genes by the activity of their products. *Science* 286, 1153-1155

26 Shull, N.P., et al. (2005) A highly specific phosphatase that acts on ADP-ribose 1"-phosphate, a metabolite of tRNA splicing in Saccharomyces cerevisiae. *Nucleic Acids Res.* 33, 650-660
27 Egloff, M.-P., et al. (2006) Structural and Functional Basis for ADP-Ribose and Poly(ADP-Ribose) Binding by Viral Macro Domains. *J. Virol.* 80, 8493-8502

28 Putics, A., et al. (2005) ADP-ribose-1″-monophosphatase: a conserved coronavirus enzyme that is dispensable for viral replication in tissue culture. *J. Virol.* 79, 12721-12731

29 Rack, J.G., et al. (2016) Macrodomains: Structure, Function, Evolution, and Catalytic Activities. *Annu. Rev. Biochem.* 85, 431-454

30 Karras, G.I., et al. (2005) The macro domain is an ADP-ribose binding module. *EMBO J.* 24, 1911-1920

31 Malet, H., et al. (2009) The Crystal Structures of Chikungunya and Venezuelan Equine Encephalitis Virus nsP3 Macro Domains Define a Conserved Adenosine Binding Pocket. *J. Virol.* 83, 6534-6545

32 Neuvonen, M. and Ahola, T. (2009) Differential Activities of Cellular and Viral Macro Domain Proteins in Binding of ADP-Ribose Metabolites. *J. Mol. Biol.* 385, 212-225

33 Xu, Y., et al. (2009) Crystal Structures of Two Coronavirus ADP-Ribose-1″-Monophosphatases and Their Complexes with ADP-Ribose: a Systematic Structural Analysis of the Viral ADRP Domain. *J. Virol.* 83, 1083-1092

34 Barkauskaite, E., et al. (2015) Structures and Mechanisms of Enzymes Employed in the Synthesis and Degradation of PARP-Dependent Protein ADP-Ribosylation. *Mol. Cell* 58, 935-946

35 Li, C., et al. (2016) Viral Macro Domains Reverse Protein ADP-Ribosylation. *J. Virol.* 90, 8478-8486

36 Fehr, A.R., et al. (2016) The Conserved Coronavirus Macrodomain Promotes Virulence and Suppresses the Innate Immune Response during Severe Acute Respiratory Syndrome Coronavirus Infection. *mBio* 7, e01721-16

37 EckeI, L., et al. (2017) The conserved macrodomains of the non-structural proteins of Chikungunya virus and other pathogenic positive strand RNA viruses function as mono-ADP-ribosylhydrolases. *Sci. Rep.* 7, 41746

38 McPherson, R.L., et al. (2017) ADP-ribosylhydrolase activity of Chikungunya virus macrodomain is critical for virus replication and virulence. *Proc Natl Acad Sci* 114, 1666-1671
39 Nan, Y., et al. (2014) Hepatitis E Virus Inhibits Type I Interferon Induction by ORF1 Products. *J. Virol.* 88, 11924-11932

40 Ojha, N.K. and Lole, K.S. (2016) Hepatitis E virus ORF1 encoded macro domain protein interacts with light chain subunit of human ferritin and inhibits its secretion. *Mol. Cell. Biochem.* 417, 75-85

41 Zoll, J., et al. (2002) The mengovirus leader protein suppresses alpha/beta interferon production by inhibition of the iron/ferritin-mediated activation of NF-kappa B. *J. Virol.* 76, 9664-9672

42 Parvez, M.K. (2015) The hepatitis E virus ORF1 ’X-domain’ residues form a putative macrodomain protein/Appr-1”-pase catalytic-site, critical for viral RNA replication. *Gene* 566, 47-53

43 Anang, S., et al. (2016) Identification of critical residues in Hepatitis E virus macro domain involved in its interaction with viral methyltransferase and ORF3 proteins. *Sci. Rep.* 6, 25133

44 Park, E. and Griffin, D.E. (2009) The nsP3 macro domain is important for Sindbis virus replication in neurons and neurovirulence in mice. *Virology* 388, 305-314

45 Foy, N.J., et al. (2013) Hypervariable domain of nonstructural protein nsP3 of Venezuelan equine encephalitis virus determines cell-specific mode of virus replication. *J. Virol.* 87, 7569-7584

46 Michel, G., et al. (2007) Adaptation of Venezuelan equine encephalitis virus lacking 51-nt conserved sequence element to replication in mammalian and mosquito cells. *Virology* 362, 475-487

47 De, I., et al. (2003) Functional Analysis of nsP3 Phosphoprotein Mutants of Sindbis Virus. *J. Virol.* 77, 13106-13116

48 Lulla, A., et al. (2012) Macromolecular assembly-driven processing of the 2/3 cleavage site in the alphavirus replicase polyprotein. *J. Virol.* 86, 553-565

49 Fehr, A.R., et al. (2015) The nsp3 Macrodomain Promotes Virulence in Mice with Coronavirus-Induced Encephalitis. *J. Virol.* 89, 1523-1536

50 Neuman, B.W. (2016) Bioinformatics and functional analyses of coronavirus nonstructural proteins involved in the formation of replicative organelles. *Antiviral Res.* 135, 97-107
51 Tan, J., et al. (2009) The SARS-unique domain (SUD) of SARS coronavirus contains two macrodomains that bind G-quadruplexes. *PLoS Pathog* 5, e1000428

52 Kusov, Y., et al. (2015) A G-quadruplex-binding macrodomain within the “SARS-unique domain” is essential for the activity of the SARS-coronavirus replication–transcription complex. *Virology* 484, 313-322

53 Eriksson, K.K., et al. (2008) Mouse hepatitis virus liver pathology is dependent on ADP-ribose-1'-phosphatase, a viral function conserved in the alpha-like supergroup. *J. Virol.* 82, 12325-12334

54 Kuri, T., et al. (2011) The ADP-ribose-1'-monophosphatase domains of severe acute respiratory syndrome coronavirus and human coronavirus 229E mediate resistance to antiviral interferon responses. *J. Gen. Virol.* 92, 1899-1905

55 Daniels, C.M., et al. (2014) Phosphoproteomic approach to characterize protein mono- and poly(ADP-ribosyl)ation sites from cells. *J Proteome Res* 13, 3510-3522

56 Grady, S.L., et al. (2012) Herpes simplex virus 1 infection activates poly(ADP-ribose) polymerase and triggers the degradation of poly(ADP-ribose) glycohydrolase. *J. Virol.* 86, 8259-8268

57 Child, S.J., et al. (1988) Inhibition of vaccinia virus replication by nicotinamide: evidence for ADP-ribosylation of viral proteins. *Virus Res.* 9, 119-132

58 Tempera, I., et al. (2010) Regulation of Epstein-Barr virus OriP replication by poly(ADP-ribose) polymerase 1. *J. Virol.* 84, 4988-4997

59 Yamada, T., et al. (2016) Constitutive aryl hydrocarbon receptor signaling constrains type I interferon-mediated antiviral innate defense. *Nat. Immunol.* 17, 687-694

60 Guo, X., et al. (2004) The zinc finger antiviral protein directly binds to specific viral mRNAs through the CCCH zinc finger motifs. *J. Virol.* 78, 12781-12787

61 Kuny, C.V. and Sullivan, C.S. (2016) Virus-Host Interactions and the ARTD/PARP Family of Enzymes. *PLoS Pathog* 12, e1005453

62 Liu, C.H., et al. (2015) Battle between influenza A virus and a newly identified antiviral activity of the PARP-containing ZAPL protein. *Proc Natl Acad Sci U S A* 112, 14048-14053

63 Atasheva, S., et al. (2014) Interferon-stimulated poly(ADP-Ribose) polymerases are potent inhibitors of cellular translation and virus replication. *J. Virol.* 88, 2116-2130
64 Atasheva, S., et al. (2012) New PARP gene with an anti-alphavirus function. *J. Virol.* 86, 8147-8160

65 Bueno, M.T., et al. (2013) Poly(ADP-ribose) polymerase 1 promotes transcriptional repression of integrated retroviruses. *J. Virol.* 87, 2496-2507

66 Daugherty, M.D., et al. (2014) Rapid Evolution of PARP Genes Suggests a Broad Role for ADP-Ribosylation in Host-Virus Conflicts. *PLoS Genet.* 10, e1004403

67 Nan, Y. and Zhang, Y.-J. (2016) Molecular Biology and Infection of Hepatitis E Virus. *Front Microbiol.* 7, 1419

68 Kamar, N., et al. (2014) Hepatitis E virus infection. *Clin. Microbiol. Rev.* 27, 116-138

69 Parkman, P.D. (1996) Togaviruses: Rubella Virus. In *Medical Microbiology* (4th edn) (Baron, S., ed)

70 Schmaljohn, A.L. and McClain, D. (1996) Alphaviruses (Togaviridae) and Flaviviruses (Flaviviridae). In *Medical Microbiology* (4th edn) (Baron, S., ed)

71 Vijay, R. and Perlman, S. (2016) Middle East respiratory syndrome and severe acute respiratory syndrome. *Curr Opin Virol* 16, 70-76

72 Song, D., et al. (2015) Porcine epidemic diarrhea: a review of current epidemiology and available vaccines. *Clin Exp Vaccine Res* 4, 166-176
Figure Legends

Figure 1. Removal of ADP-ribose From Protein by Macrodomains. A schematic representation of de-ADP-ribosylation reaction of proteins modified on acidic residues. Proximal and distal ribose are shaded in gray, α and β phosphate groups are shaded in orange.

Figure 2. Structures and Multiple Sequence Alignment of Viral Macrodomains. (Top left) A conserved surface representation of SARS macrodomain (PDB:2FAV) based on ConSurf analysis. The most conserved residues are coloured in magenta, while the least conserved residues are coloured in cyan. ADP-ribose is shown in stick representation. (Top right) ADP-ribose binding cleft of SARS macrodomain alone (gray) (PDB:2FAV), aligned with CHIKV macrodomain (Middle left, orange) (PDB:3GPO), or human MacroD2 macrodomain (Middle right, salmon) (PDB:4IQY). The proteins are shown as cartoon, with key residues shown as sticks. Loop 1 and loop 2 are coloured in slate and pale-green, respectively (SARS-CoV macrodomain only). (Bottom) Sequence alignment of selected viral and human macrodomains. The name of the virus is indicated on the left. Structure availability is indicated by a PDB code; for viral macrodomains where no structural information is available, UniProt retrieved sequences of the macrodomains were used and are indicated as (Seq.). Secondary structure elements are schematically depicted above the alignment and the numbering is for a generic macrodomain (i.e. not including additional helices or sheets present in some but not all macrodomains). Asterisks and red boxes indicate highly conserved, mostly substituted or key catalytic determinant residues of viral macrodomains. Magenta shaded boxes depict the degree of conservation.

Figure 3. Genomic Location of Viral Macrodomains. Diagram depicting the genomic locations of macrodomains in the Hepeviridae, Togaviridae, and Coronaviridae. All viral
macromdomains are a distinct domain of a larger non-structural protein, ORF1 in Hepeviridae and
nsP3 in both the Togaviridae and Coronaviridae. For Coronaviridae there are multiple
macromdomains, listed Mac1/2/3. Mac1 is analogous to macromdomains found in other viruses.
Table 1. Representative mutations to that disrupt viral macrodomain function and their effects in vivo.

| Family       | Virus    | Mutations | ARH act. | ADRP act. | In vivo effect | Ref.       |
|--------------|----------|-----------|----------|-----------|---------------|------------|
| Coronaviridae| SARS-CoV  | wt        | ++       | ++        |               | [27, 35, 36]|
|              |          | D23A      | +        |           | ↓ Virulence   | [36]       |
|              |          | N38A      | +/-      |           |               | [27]       |
|              |          | N41A      | -        | -         | ↓ Virulence   | [27, 36]  |
|              |          | H46A      | +/-      | +/-       | ↓ Virulence   | [27, 36]  |
|              |          | G131V     | -        |           | ↓ Virulence   | [36]       |
|              |          | G47A+G48A | +/-      |           |               | [27, 36]  |
|              |          | F133A     | -        |           |               | [27]       |
|              | HCoV229E | wt        | ++       | ++        |               | [28, 35]  |
|              |          | N28A      | ++       |           |               | [28]       |
|              |          | N37A+N40A | -        |           |               | [28]       |
|              |          | N37A      | -        |           |               | [28]       |
|              |          | N40A      | -        |           |               | [28]       |
|              |          | H45L      | -        |           |               | [28]       |
|              |          | G47A | V | +/- | - |               | [28]       |
|              |          | G48A | V | +/- | - |               | [28]       |
|              | FIPV     | wt        | ++       |           |               | [37]       |
|              |          | MHV       | N30A     |           | ↓ Virulence & Replication | [49, 53] |
| Hepeviridae  | HEV      | wt        | ++       | ++        |               | [27, 35, 37]|
|              |          | N38A      |          |           | ↓ Replication | [42]       |
|              |          | N42A      | +/-      |           | Not viable | ↓ Replication | [35, 42] |
|              |          | H45A      |          |           | Not viable |               | [42]       |
|              |          | G48A | V |   |   | ↓ Replication | no effect | [42]       |
|              |          | G49A | V |   |   | Not viable |               | [42]       |
|              |          | G50A | V | +/- | - | ↓ Replication | not viable | [35, 42] |
|              |          | G48S+G49S |    |   | ↓ Replication |               | [35]       |
|              |          | G48S+G49S+G50A |    |   | ↓ Replication |               | [35]       |
|              |          | G123A     | +/-      |           | ↓ Replication |               | [35]       |
|              |          | I124A     | +        |           |               | [35]       |
|              |          | Y125F     | +        |           |               | [35]       |
|              |          | Y125A     |          | (misfold) |               | [35]       |
| Togaviridae  | CHIKV    | wt        | ++       | ++        |               | [31, 37, 38]|
|              |          | D10A      | +/-      | +         | Reverted to WT | [31, 38] |
|              |          | N24A      | +/-      | +/-       |               | [31, 37]  |
|              |          | N24R | Y | - | - |               | [37]       |
|              |          | G32E | D | Q | - | Reverted to WT (G32E) | [38]       |
| Mutation | Virulence & Replication |
|----------|-------------------------|
| G32A | S | + | +/- | ↓ Virulence & Replication [38] |
| V33A | F | +/- | [37] |
| V33E | - | [37] |
| T111A | + | ↓ Virulence & Replication [38] |
| G112E | +/- | Reverted to WT [38] |
| Y114A | +/- | - | ↓ Virulence & Replication [31, 37, 38] |
| Y114V | W | +/- | [37] |
| R144A | ++ | Reverted to WT [38] |
| G32E+V113R+Y114N | - | Reverted to G32A+V113R+Y114N or V113R+V114N [38] |
| G32A+V113R+Y114N | +/- | [38] |
| V113R+Y114N | ++ | [38] |
| SFV | wt | + | [32] |
| D10A | - | [32] |
| N21A+N24A | - | [32] |
| D31G | +/- | [32] |
| G32Y | - | [32] |
| G112Y | - | [32] |
| SINV | wt | ++ | [37] |
| N10A | ↓ Virulence & Replication [44] |
| N24A | ↓ Virulence [44] |
| N10A+N24A | ↓ Virulence & Replication [44] |
| VEEV | wt | ++ | [35, 37] |
| ONNV | wt | ++ | [37] |

554  
555  "ADP-ribose hydrolase activity  
556  "ADP-ribose-1"-phosphatase activity  
557  " - no activity; +/- minimal activity; + modest activity; ++ robust activity  
558  "HCoV-229E – human coronavirus 229E  
559  "FIPV – feline infectious peritonitis virus  
560  "VEEV – Venezuelan equine encephalomyelitis virus  
561  "ONNV – O'nyong’nyong virus  
562  
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OUTSTANDING QUESTIONS

Do viral macrodomains counteract antiviral ADP-ribosylation? Which PARP(s) is responsible for antiviral ADP-ribosylation? Do invertebrates have anti-viral PARP activity that is counteracted by macrodomains?

What are the ADP-ribosylated protein targets of viral macrodomains? Are targets mono- or poly-ADP-ribosylated? How does ADP-ribosylation impact these targets? What are the determinants of mono- vs poly-ADP-ribose binding/removal? Do all viruses require macrodomain enzymatic activity or is MAR/PAR binding sufficient to promote virus fitness/virulence? What effects do mutations in viral macrodomains have on de-ADP-ribosylation kinetics?

How do viral macrodomains acquire specificity? Do regions outside of the ADP-ribose binding pocket contribute to interactions with targets? Can a macrodomain from one virus family substitute for a different one?

Why are macrodomains encoded by some but not all virus families? Does antiviral ADP-ribosylation occur in other virus infections? Do other viruses interfere with PARP activity and by what mechanisms?

Is the development of small molecule inhibitors specific for viral macrodomains to repress viral pathogenesis feasible? Could viruses escape these inhibitors? Would these inhibitors also affect mammalian enzymes, leading to significant side effects?
**Trends**

Macrodomains were discovered in Togaviruses, Coronaviruses, and the Hepatitis E virus over 25 years ago. They were called the “X” domain because they had no known function.

About 10 years later, several macrodomain structures were determined. They consist of central β-sheets surrounded by α-helices and bind to ADP-ribose and its derivatives. They were named after the structurally homologous domain of the MacroH2A histone.

Originally described as ADP-ribose-1′′-phosphatases, both cellular and viral macrodomains enzymatically remove mono- and poly-ADP-ribose from proteins, supporting the notion that protein ADP-ribosylation is a component of the antiviral response.

Chikungunya virus and Hepatitis E virus macrodomains are critical for replication, while the Coronavirus macrodomain both blocks the innate immune response and separately promotes *in vivo* replication.
| Protein | Accession | Sequence  |
|---------|-----------|-----------|
| SARS   | 2ACF      | KCVDETVKEAQSANPMVIVNAANLHKGGSVAGALKN56...122ILLLAPLLSAGIFGAKPLQ139 |
| MERS   | 3DUS      | VILDAIQYAKGYESVILVNAANTHLKHHGIAGAINA36...122LVVTPLVYSAGIFGKPAV159 |
| 229E   | 3EJG      | YOGDVTDDVNGVDGFVILVNAANENLAAAGGGLAKALDV55...119TPLDILSCGIFGKILET136 |
| FCov   | 3ETI      | YQGDLDVLIINFEPDVILVNAANGDLREVGVARAIDV56...123KILTPLISVGIFKVKLEV140 |
| MHV    | (Seq.)    | VKGDAVIVKVRVNVNEVIAVNPANGRMAGAAGVAGAIAE44...112NVTTTLISAGIFSVPDTVD129 |
| CHIKV  | 3GPG      | KRMDIANK...-NDEECVNAANPRGLPGCKAVYK39...105SVAILLLSTGVYSGGKDR120 |
| VEEV   | 3GQE      | VRGDIAT...-ATEGIVENANSKQPGGVCGLAYK39...103SVAILLSTGIFSGNKDR120 |
| SFV    | (Seq.)    | KRADDIAV...-CTEAAYVNAVARGTVGVDVRCAYK39...103SVAILLSTGVFSGGDR120 |
| SINV   | 4GU4      | KRENIA...-CQELAYVNAENPLGRPCGCVRAVYK39...103SVAILLSTGTYAACGDR120 |
| HEV    | (Seq.)    | FAGSLFE...-STCTWLVNASNDHRPGGGLCHAFYQ57...114TAAYPLLGVTGYQVPIGP131 |
| HsD2   | 4IQY      | YRGDITL...-LEVDAAVNASLLGGGVDGCTHR109...179SVAFPC1STGITYGFPNEP196 |
Hepeviridae (Hepatitis E Virus)

Togaviridae (Sindbis virus)

Coronaviridae (SARS-CoV)