www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2021; 10(1): 212-216

www.thepharmajournal.com
Received: 21-11-2020
Accepted: 25-12-2020

Manikandan R
Ph.D. Scholar, Division of Veterinary Microbiology, ICAR-IVRI, Izatnagar, Uttar Pradesh, India

Bindu S
Ph.D. Scholar, Division of Veterinary Microbiology, ICAR-IVRI, Izatnagar, Uttar Pradesh, India

Dinesh M
Ph.D. Scholar, Division of Pathology, ICAR-IVRI, Izatnagar, Uttar Pradesh, India

Jigarji C Thakor
Ph.D. Scholar, Division of Pathology, ICAR-IVRI, Izatnagar, Uttar Pradesh, India

S Shanmuganathan
Ph.D. Scholar, Division of Virology, ICAR-IVRI, Izatnagar, Uttar Pradesh, India

Gollapalli Sivarama Krishna
Assistant Professor, Department of Veterinary Microbiology, College of Veterinary Science, Garividi, Tirupati, Andhra Pradesh, India

Corresponding Author:
Manikandan R
Ph.D. Scholar, Division of Veterinary Microbiology, ICAR-IVRI, Izatnagar, Uttar Pradesh, India

Viral miniproteins: Immunobiology- A special emphasis on RNA viruses role in replication and virulence

Manikandan R, Bindu S, Dinesh M, Jigarji C Thakor, S Shanmuganathan and Gollapalli Sivarama Krishna

Abstract
Viruses encode short transmembrane proteins that play vital roles in virus replication or virulence. These viral miniproteins are often short less than 100 amino acids. Some of these proteins oligomerize in membranes and form ion channels (viroporins). Viroporins are small proteins with at least one amphipathic helix that constitutes its transmembrane domain, spanning lipid membranes. Viroporins induced host responses are activation of inflammasome, apoptosis, and autophagy. Viroporins place an important role in viral entry, uncoating, replication, assembly and release in the viral life cycle. Most of the viral mini proteins were discovered by viral genome sequencing. The rational design of specific antiviral drugs can also be formulated against the viral miniproteins which play a very integral role in the viral lifecycle. Further scientific research strategies are required to counteract the immunobiology mediated by the viral miniproteins and may also for discovering the mutants lacking viral miniproteins attenuated vaccines.

Keywords: Viral miniproteins, viroporins, viral replication, antiviral drugs

Introduction
Viruses codes for very short transmembrane proteins that play integral roles in virus replication and virulence. These viral mini proteins are often 50-100 amino acids and not homologous to cellular proteins, their open reading frames were often overlooked during the initial annotation of viral genomes. Some of these viral mini proteins oligomerize in membranes and form ion channel activity. Some other mini proteins bind to cellular transmembrane proteins and modulate the activity, whereas still others have an unknown mechanism of action. Miniproteins are not restricted to RNA or DNA viruses or a particular viral genome replication strategy. Even though these viral encoded short transmembrane proteins are called mini proteins but also play a very major and vital role in replication and virulence of the virus. This present review focuses mainly on what the viral mini proteins can induce pathophysiology to the host, host immune response against the mini proteins to evade and antiviral strategies to inhibit the viral mini proteins.

Functions of miniproteins
Viral Miniproteins act as a Viroporins like activity. viroporins are small, hydrophobic trans-membrane viral proteins that oligomerize to form hydrophilic pores in the host cell membranes [1].

- The ion channel activity of viroporin causes disruption in the cellular ion homeostasis, in particular the calcium ion. Fluctuation in the calcium level triggers the activation of the host defensive programmed cell death pathways as well as the inflammasome, which in turn are being subverted for the virus replication benefits [2].
- These proteins are crucial for the pathogenicity and replication of viruses as they aid in various stages of the viral life cycle from genome uncoating to viral release.
- Several viroporins have also been shown to localize to the endoplasmic reticulum (ER) and/or its associated membranous organelles. Replication of most RNA viruses is closely linked to the ER and has been found to cause ER stress in the infected cells [3]. On the other hand, autophagy is an evolutionarily conserved “self-eating” mechanism that is also observed in cells infected with RNA viruses.
- Both ER stress and autophagy are also known to modulate a wide variety of signaling

pathways including pro-inflammatory and innate immune response, thereby constituting a major aspect of host-virus interactions [3].

- It is possible to build artificial small transmembrane proteins called traptamers. Traptamers modulate a variety of biological processes [4].

Classification

Recent categorization places viroporins into two major classes based on the number of transmembrane domain (TMD) that are then further classified into A or B subclasses based on their membrane topology. Single TMD viroporins in subclass A have their N terminus facing the ER lumen while those in subclass B have their C-terminal tails in the ER lumen. For Class IIA and IIB viroporins, both N- and C-terminus are inside the ER lumen or the cytoplasmic matrix, respectively. An additional third class of viroporins may be necessary as viroporins with three-pass TMD have been proposed, such as the non-structural protein 4 (NSP4) of rotavirus [5] and 3a of severe acute respiratory syndrome SARS-CoV [6].

Structure

Recent advancement in technology such as the ability to characterize protein structure using nuclear magnetic resonance (NMR) spectroscopy, has successfully resolved the structure of several viroporins. X-ray crystallographic images can also be used to resolve the structure of viroporins transmembrane domain. M2 of IAV (Influenza Type A Virus) forms a tetrameric pore on the plasma membrane that adopts different conformations as it conducts proton across the membrane, whereas for p7 of HCV(Hepatitis C virus), a hexameric flower-shaped complex was revealed via single-particle electron microscopy. P7 has also been found to exist in heptameric form using transmission electron microscopy and a model of how both forms could coexist was proposed [7].

Viroporins induced host response

During an active infection, a virus hijacks the host molecular machinery and turns the host cell into a virus factory. When the ion channel activity of viroporin is activated during viral infection, perturbation of the host membrane permeability occurs, leading to disruption of the cellular ion homeostasis and subsequent cytopathic events. Many viroporins are expressed as endoplasmic reticulum (ER) proteins and remained localized to the ER during virus replication, while others are partially localized to the mitochondria [8]. The ER serves as the organelle for the cell's calcium ions storage. The expression of viral ion channels at the ER membrane causes leakage of Ca2+ from the ER storage into the cytoplasm. It can also trigger several defensive signaling pathways, including apoptosis, autophagy, and inflammasome formation, in an attempt to contain and eliminate the invader. However, viruses have evolved to adapt and subvert these defense mechanisms for their growth benefits [2].

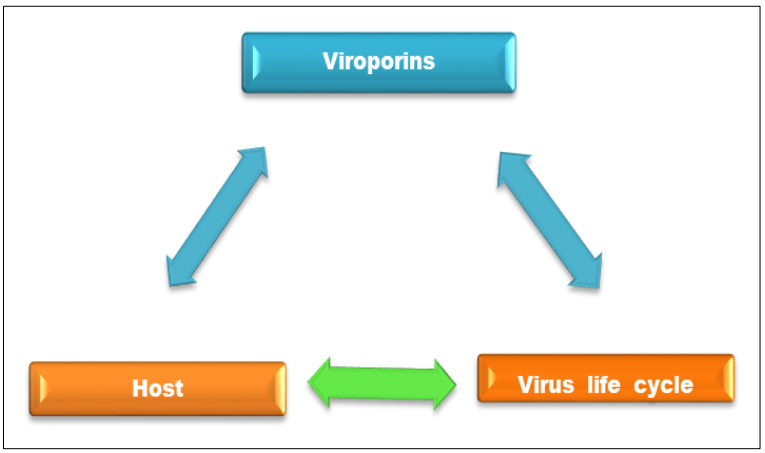


Fig 1: Dual channel role of viroporins in host defense signaling pathway and viral replication

Apoptosis

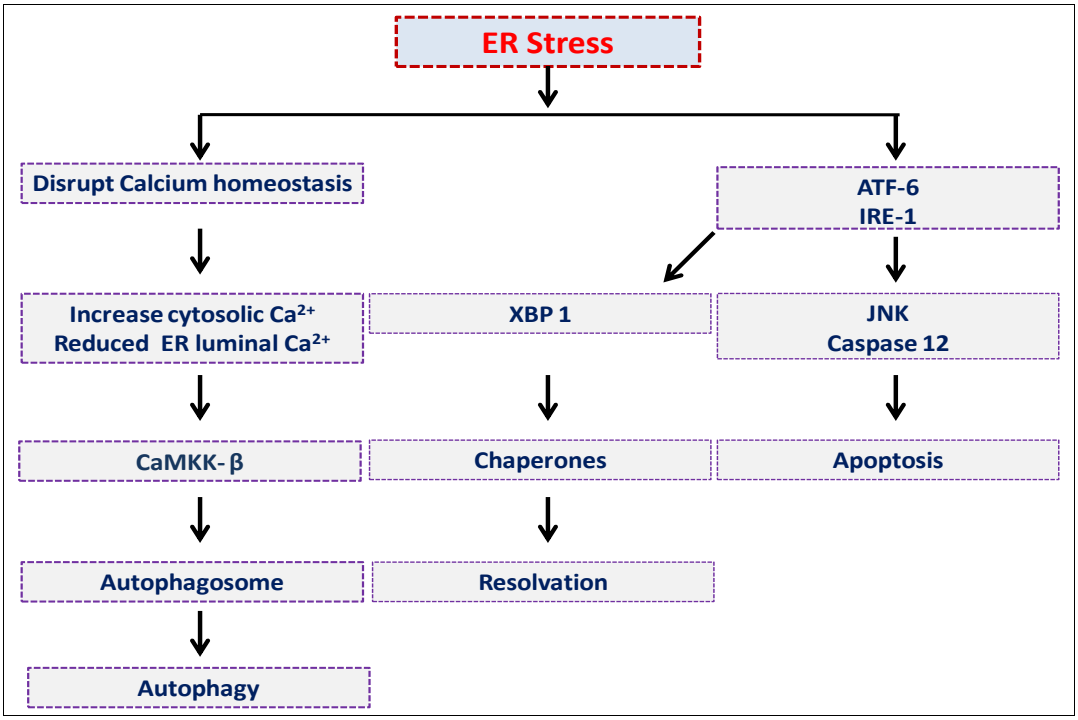
Apoptosis is a genetically programmed cellular death mechanism used by the host to eliminate damaged or unwanted cells through the activation of the caspase cascade. There are two main signaling pathways, the extrinsic or receptor-mediated pathway, and the intrinsic or mitochondrial pathway. Perturbation of the ER Ca2+ storage can also activate an ER-specific apoptotic pathway that ultimately leads to the activation of the common apoptosis effector caspase, caspase-3 [9]. Viroporins from several viruses have been shown to trigger apoptosis in a caspase-dependent manner but the mechanism involved differs between each virus. Expression of several viroporins from RNA viruses including the 6K protein from Sindbis virus (SV; family *Togaviridae*), M2 from influenza A virus (IAV; family *Orthomyxoviridae*), and 2B and 3A from poliovirus (PV; family *Picornaviridae*) can activate caspase-3 and lead to the release of cytochrome-c from the mitochondria proving the ability of these viroporins to activate the intrinsic programmed cell death pathway [8].

Autophagy

Autophagy occurs through the intracellular membrane trafficking system by delivering damaged or unwanted cellular material from the cytosol to the lysosome for degradation, and thus can be referred to as "self-eating". During autophagy, cellular components are sequestered into a double-membrane vesicle termed an autophagosome, which then fuses with a lysosome where degradation can occur. Viruses have evolved to manipulate and interfere with different stages of this destructive process to their benefit.

For instance, M2 from the influenza virus can inhibit the fusion between autophagosome and lysosome independent of its proton channel activity by interacting with the autophagy-related protein, Atg6/Beclin-1 [10]. By doing so, the degradation of the autophagosomes is inhibited, leading to the accumulation of these vesicles in the infected cells. Some viral infections can induce autophagy-related vesicles'

formation so they can be subverted to the viruses' advantage. For rotavirus infection, autophagy is induced when NSP4 permeabilizes the ER membrane and releases the calcium storage into the cytoplasm. Further, it activates the calcium-dependent autophagy pathway, which is then tricked into transporting the viral proteins from the ER to the viroplasm for replication and assembly [11]. During SARS-CoV infection, the virus has a profound impact on ER function and hijacks the ER to process its structural and nonstructural proteins. When the damage to the ER is severe, the unfolded protein response (UPR) triggers apoptosis. To combat that SARS-CoV activates three key proximal sensors of the UPR, activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and PKR-like ER kinase (PERK), they are resident transmembrane proteins of ER and it further governs UPR signaling to activate different genes of encoding ER chaperones, folding enzymes, and other proteins required for protein folding, maturation, and degradation. SARS-CoV mini proteins modulate the UPR to facilitate viral replication [12].



[CaMKK: Calcium/calmodulin dependent kinases kinase-β, ATF-6: activation of the transcriptional factor-6, IRE-1: inositol-requiring enzyme 1, JNK: c-Jun N-terminal kinase, XBP1: X-box protein 1]

Fig 2: Signaling pathway mechanism of viroporins induced ER stress

Inflammasomes

The antiviral innate immune response that is activated from the host during ion flux is the complex termed the "inflammasome." An inflammasome is a caspase-activating complex that upon induction results in caspase-1 activation and pro-inflammatory cytokines secretion. One of the inflammasome complexes that can be activated via disturbances in intracellular ionic concentration in addition to the pathogen-associated molecular patterns (PAMPs) is the Nod-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome. Several respiratory viruses are known to activate the NLRP3 inflammasome in the lung during infection by causing disturbances to the cellular potassium and calcium ion homeostasis via viroporins. IAV is the most common activator of the NLRP3 inflammasome, in which it induces the maturation of caspase-1 via dsRNA and altering the ionic concentration leads to the subsequent secretion of the pro-inflammatory cytokine, such as IL-1 [13].

Major viral miniproteins of RNA viruses

Influenza virus – M2 protein

Influenza viruses, enveloped viruses with segmented RNA genomes, are of great medical importance. Influenza A virus encodes the M2 protein (A/M2), a 97-amino-acid integral membrane phosphoprotein with a single membrane-spanning domain and a 54-amino-acid carboxy-terminus cytoplasmic tail [14]. A tetramer of M2 exists in cell membranes and the envelope of the virus particle, where it acts as a proton-selective ion channel. The vast majority of these channel inhibitors have been developed against the influenza A virus M2 (IAV M2 or AM2), which is the first viroporin discovered [15].

M2 proton channel activity is essential for influenza virus replication. Internalization of the influenza virion deposits it in the endosome, but virus uncoating and delivery of the viral genome into the cytoplasm requires acidification of the interior of the virion. Virion acidification is mediated by the proton channel activity of the M2 tetramer, which conducts protons from the endosomal lumen into the virion interior. The ion channel is acid gated highly selective for H^+ ions (not voltage-gated) [7].

Mutational analysis and biochemical studies identified the crucial amino acids that mediate M2 channel activity, including two polar amino acids on a single turn of the transmembrane α-helix, histidine 37 and tryptophan 41 [16]. Replacing the histidine with any of several amino acids allows M2 to transport Na^+ and K^+ as well as H^+, showing that histidine confers ion selectivity. The tryptophan appears to play an important role in activating the channel in response to low pH, by participating in a pH-dependent conformation change [17].

A/M2 is the target of the antiviral drug amantadine and

rimantadine, which block virus uncoating by inhibiting the proton channel activity of M2. Mutations in M2 can cause amantadine resistance. Recent studies demonstrated that amantadine binds inside the aqueous pore of the M2 tetramer and inhibits proton channel activity by physically obstructing ion flow leads to inhibition of uncoating [18, 19].

Paramyxovirus - SH proteins

The *Paramyxoviruses* are enveloped RNA viruses, which include important human pathogens such as mumps virus and respiratory syncytial virus. Many *paramyxoviruses* encode a short hydrophobic (SH) integral membrane virion protein between 44 and 60 amino acids long. SH proteins are not required for virus replication in cultured cells, but viruses lacking the SH gene are attenuated *in vivo* [20]. Notably, the SH proteins of respiratory syncytial virus and parainfluenza virus 5 are interchangeable *in vivo*, despite marked sequence differences [21].

Recent studies found out that particularly striking features of mumps virus SH is a type I transmembrane protein, i.e., with its carboxy-terminus in the cytoplasm, whereas SV5 SH displays the opposite orientation. *Paramyxoviruses* lacking the SH protein display enhanced cytopathic effect and induction of apoptosis, suggesting that a major physiologic role of the SH protein is the inhibition of apoptosis induced by viral replication [22]. Human metapneumoviruses lacking the SH protein induce enhanced secretion of pro-inflammatory mediators in the airway of infected mice as a consequence of increased activation of the transcription factor NF- κB [23].

Picorna virus - 2B

Poliovirus - 2B

Protein 2B is a -100 residues long nonstructural protein found in enterovirus, eg, *poliovirus*. The single open reading frame encodes a peptide chain divided into three regions, P1 to P3. One of the non-structural proteins from the polypeptides in the P2 region. Poliovirus -2B. Two TM domains, one is more hydrophobic than the other and found to increase the concentrations of free calcium in the cytosol. In *poliovirus*, 2B and 3A viroporins can inhibit the cellular protein secretion pathway by disassembling the Golgi complex or by blocking the ER–Golgi trafficking, which results in the accumulation of membrane vesicles in the cytoplasm. By interfering with the secretory pathway, poliovirus thwarts the host immune response against viral infection by shutting off the nascent MHC class I trafficking as well as down-regulating cytokine release. It has been proposed to promote viral replication and repression of the antiviral immune response of host cells [24].

FMDV- 2B

Foot-and-mouth disease virus (FMDV), a single-stranded, positive RNA virus with a genome of approximately 8,500 bases, belongs to the *Aphthovirus* genus of the *Picornaviridae* family. Nonstructural protein 2B of foot-and-mouth disease (FMD) virus (FMDV) is comprised of a small, hydrophobic, 154-amino-acid protein. Structure-function analyses demonstrated that FMDV 2B is an ion channel-forming protein. 2B can insert itself into cellular membranes to form a pore. This pore allows the passage of ions and small molecules through the membrane. They also discovered for the first time a virus with a pore-forming protein that contains two independent functional pores. By making mutations in the infectious clone of FMDV, they determined that mutations in either pore resulted in the non-viable virus. Alterations to any of these structures interfered with pore channel activity and the capacity of the protein to permeabilize the endoplasmic reticulum (ER) to calcium and were lethal for virus replication. Thus, FMDV-2B emerges as the first member of the viroporin family containing two distinct pore domains. This suggests that both pore-forming functions are independently required during FMDV infection and further stated as essential for the viral replication [25].

BTV- NSP3

Bluetongue virus (BTV) is a member of the *Orbivirus* genus within the *Reoviridae* family of segmented double-stranded RNA viruses. Viroporins have been implicated in promoting virus release from infected cells and in affecting cellular functions including protein trafficking and membrane permeability. Nonstructural protein 3 (NSP3) of bluetongue virus has been shown previously to be important for the efficient release of newly made virions from infected cells. NS3 localizes to the Golgi apparatus and plasma membrane in transfected cells and it can homo-oligomerize in transfected cells. Usually, BTV is canonically released by cell lysis only but it also exits non-lyrically. In infected cells, the BTV nonstructural glycoprotein 3 (NSP3) is found to be associated with host membranes and traffics from the endoplasmic reticulum through the golgi apparatus to the plasma membrane. This suggests a role for NSP3 in BTV particle maturation and non-lytic egress. They demonstrated that correct trafficking of the NSP3 protein is required for virus maturation and release with the help of two polybasic motifs (PMB1/PMB2) [26].

SARS - CoV- 3a, E and 8a

Severe acute respiratory syndrome coronavirus (SARS-CoV), a member of the genus *Betacoronavirus* within the family *Coronaviridae*, is an enveloped virus with a single-stranded positive-sense RNA genome of approximately 30 kb in length. The genome encodes four structural proteins, spike (S), envelope (E), matrix (M) and nucleocapsid (N), and non-structural proteins, along with a set of accessory proteins (3a, 3b, 6, 7a, 7b, 8a, 8b, and 9b) [27]. SARS-CoV is the etiological agent of SARS [28]. While many coronaviruses (CoVs) encode two viroporins, severe acute respiratory syndrome CoV (SARS-CoV) encodes three: proteins 3a, E, and 8a. They showed that the full-length E and 3a proteins were required for maximal SARS-CoV replication and virulence, whereas viroporin 8a had only a minor impact on these activities. A virus missing both the E and 3a proteins was not viable, whereas the presence of either protein with a functional PDZ-binding motif (PBM) restored virus viability. Collectively, these results demonstrate key roles for the ion channel and PBM domains in optimal virus replication and pathogenesis and suggest that the viral viroporins and PBMs are suitable targets for antiviral therapy and mutation in attenuated SARS-CoV vaccines [29].

Conclusions

Most viral mini proteins were discovered by viral genome sequencing. Many viruses encode small transmembrane proteins, some shorter than 50 amino acids. Viral mini proteins regulate many aspects of viral replication and cell physiology. Viroporins, one class of mini proteins, oligomerize and form ion channels. A striking feature of viral mini proteins is the relatively flexible sequence requirements

in the transmembrane domain. Viral mini proteins and traptamers are having high specificity. Artificial small transmembrane proteins that display a variety of biological activities can be constructed and selected. Artificial small transmembrane Proteins modeled on viral mini proteins called Traptamer protein-Can alter cell behavior. Traptamers can also be used to activate/dissect cellular pathways. Ribosome profiling, which has identified novel translated ORFs in cytomegalovirus, will also be useful for identifying new mini proteins. Proteomic analysis of infected cells or purified virions may be useful in future discovery. Future research strategies may also overlook most of the nonstructural proteins showing viroporins like activity of viral mini proteins instead of looking only for the structural proteins of the virus. Rational design of specific antiviral drugs can also be formulated against the viral mini proteins which play a very integral role in viral replication, pathogenesis, and viral release. Further scientific research methodologies required to counteract the immunobiology mediated by the viral mini proteins and may also for discovering the mutants lacking viral mini proteins attenuated vaccines.

References

1. Nieva JL, Madan V, Carrasco L. Viroporins: structure and biological functions. Nat Rev Microbiol 2012;10:563-574.
2. Sze C, Tan YJ. Viral membrane channels: Role and function in the virus life cycle. Viruses 2015;7:3261-3284.
3. Fung, To Sing, Jaume Torres, Ding Xiang Liu. The emerging roles of viroporins in ER stress response and autophagy induction during virus infection. Viruses 2015;7:2834-2857.
4. DiMaio D. Viral miniproteins. Annu Rev Microbiol 2014;68:21-43.
5. Hyser JM, Collinson-Pautz MR, Utama B, Estes MK. Rotavirus disrupts calcium homeostasis by NSP4 viroporin activity. MBio 2010;1:00265-10.
6. Zeng R, Yang RF, Shi MD *et al*. Characterization of the 3a protein of SARS-associated coronavirus in infected vero E6 cells and SARS patients. J Mol Biol 2004;341:271-279.
7. Wang J, Qiu JX Soto C, DeGrado WF. Structural and dynamic mechanisms for the function and inhibition of the M2 proton channel from influenza A virus. Curr Opin Struct Biol 2011;21:68-80.
8. Madan V, Castello A, Carrasco L. Viroporins from RNA viruses induce caspase-dependent apoptosis. Cell Microbiol 2008;10:437-451.
9. Morishima N, Nakanishi K, Takenouchi H, Shibata T, Yasuhiko Y. An endoplasmic reticulum stress-specific caspase cascade in apoptosis cytochrome c-independent activation of caspase-9 by caspase-12. J Biol Chem 2002;277:34287-34294.
10. Aweya JJ, Mak TM, Lim SG, Tan YJ. The p7 protein of the hepatitis C virus induces cell death differently from the influenza A virus viroporin M2. Virus Res 2013;172:24-34.
11. Crawford SE, Hyser JM, Utama B, Estes MK. Autophagy hijacked through viroporin-activated calcium/calmodulin-dependent kinase kinase-beta signaling is required for rotavirus replication. Proc Natl Acad Sci 2012;109:E3405-E3413.
12. Chan CP, Siu KL, Chin KT, Yuen KY, Zheng B, Jin DY. Modulation of the unfolded protein response by the severe acute respiratory syndrome coronavirus spike protein. J Virol 2006;80:9279-9287.
13. Nieto-Torres JL, Verdia-Baguena C, Castano-Rodriguez C, Aguilella VM, Enjuanes L. Relevance of viroporin ion channel activity on viral replication and pathogenesis. Viruses 2015;7:3552-3573.
14. Pinto LH, Lamb RA. The M2 proton channels of influenza A and B viruses. J Biol Chem 2006;281:8997-9000.
15. Pielak RM, Chou JJ. Influenza M2 proton channels. Biochimica et Biophysica Acta (BBA)-Biomembranes 2011;1808:522-529.
16. Pinto LH, Dieckmann GR, Gandhi CS *et al*. A functionally defined model for the M2 proton channel of influenza A virus suggests a mechanism for its ion selectivity. Proc Natl Acad Sci 1997;94:11301-6.
17. Venkataraman P, Lamb RA, Pinto LH. Chemical rescue of histidine selectivity filter mutants of the M2 ion channel of influenza A virus. J Biol Chem 2005;280:21463-72.
18. Cady SD, Schmidt-Rohr K, Wang J, Soto CS, Degrado WF, Hong M. Structure of the amantadine binding site of influenza M2 proton channels in lipid bilayers. Nature 2010;463:689-92.
19. Jing X, Ma C, Ohigashi Y, Oliveira FA *et al*. Functional studies indicate amantadine binds to the pore of the influenza A virus M2 proton-selective ion channel. Proc Natl Acad Sci 2008;105:10967-72.
20. Wilson RL, Fuentes SM, Wang P *et al*. Function of small hydrophobic proteins of paramyxovirus. J Virol 2006;80:1700-9.
21. Fuentes S, Tran KC, Luthra P, Teng MN, He B. Function of the respiratory syncytial virus small hydrophobic protein. J Virol 2007;81:8361-66.
22. Lin Y, Bright AC, Rothermel TA, He B. Induction of apoptosis by paramyxovirus simian virus 5 lacking a small hydrophobic gene. J Virol 2003;77:3371-83.
23. Bao X, Kolli D, Liu T, Shan Y, Garofalo RP, Casola A. Human metapneumovirus small hydrophobic protein inhibits NF-κB transcriptional activity. J Virol 2008;82:8224-29.
24. Deitz SB, Dodd DA, Cooper S, Parham P, Kirkegaard K. MHC I-dependent antigen presentation is inhibited by poliovirus protein 3A. Proc. Natl. Acad. Sci 2000;97:13790-1379.
25. Gladue DP, Largo E, de la Arada I *et al*. Molecular characterization of the viroporin function of foot-and-mouth disease virus nonstructural protein 2B. J Virol 2018;92:e01360-18.
26. Labadie T, Jegouic S, Roy P. Bluetongue Virus Nonstructural Protein 3 Orchestrates Virus Maturation and Drives Non-Lytic Egress via Two Polybasic Motifs. Viruses 2019;11:1107.
27. Tan YJ, Lim SG, Hong W. Characterization of viral proteins encoded by the SARS-coronavirus genome. Antiviral Res 2005;65:69-78.
28. Drosten C, Preiser W, Gunther S, Schmitz H, Doerr HW. Severe acute respiratory syndrome: identification of the etiological agent. Trends Mol Med 2003;9:325-327.
29. Castano-Rodriguez C, Honrubia JM, Gutierrez Alvarez J, DeDiego ML, Nieto-Torres JL, Jimenez-Guardeno JM, Kochan G. Role of severe acute respiratory syndrome coronavirus viroporins E, 3a, and 8a in replication and pathogenesis. MBio 2018;9:e02325-17.