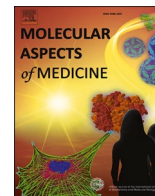




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## Dysregulation of host cell calcium signaling during viral infections: Emerging paradigm with high clinical relevance

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### ABSTRACT

Viral infections are one of the leading causes of human illness. Viruses take over host cell signaling cascades for their replication and infection. Calcium ( $\text{Ca}^{2+}$ ) is a versatile and ubiquitous second messenger that modulates plethora of cellular functions. In last two decades, a critical role of host cell  $\text{Ca}^{2+}$  signaling in modulating viral infections has emerged. Furthermore, recent literature clearly implicates a vital role for the organellar  $\text{Ca}^{2+}$  dynamics (influx and efflux across organelles) in regulating virus entry, replication and severity of the infection. Therefore, it is not surprising that a number of viral infections including current SARS-CoV-2 driven COVID-19 pandemic are associated with dysregulated  $\text{Ca}^{2+}$  homeostasis. The focus of this review is to first discuss the role of host cell  $\text{Ca}^{2+}$  signaling in viral entry, replication and egress. We further deliberate on emerging literature demonstrating hijacking of the host cell  $\text{Ca}^{2+}$  dynamics by viruses. In particular, a variety of viruses including SARS-CoV-2 modulate lysosomal and cytosolic  $\text{Ca}^{2+}$  signaling for host cell entry and replication. Moreover, we delve into the recent studies, which have demonstrated the potential of several FDA-approved drugs targeting  $\text{Ca}^{2+}$  handling machinery in inhibiting viral infections. Importantly, we discuss the prospective of targeting intracellular  $\text{Ca}^{2+}$  signaling for better management and treatment of viral pathogenesis including COVID-19. Finally, we highlight the key outstanding questions in the field that demand critical and timely attention.

### 1. Introduction

Viral infections are one of the most common causes of human illness worldwide (Burrell et al., 2017; Parvez and Parveen, 2017). Several viral infections are endemic in nature while some others can lead to pandemic diseases such as novel coronavirus disease (COVID-19). Viruses utilize host cell molecular machinery for driving their replication and dissemination. Therefore, virus-host cell interaction and subsequent modulation of host cell signaling pathways is a hallmark of viral pathogenesis (Rouse and Sehrawat, 2010). One of the most commonly dysregulated host cell signaling cascades during viral infections is cellular calcium ( $\text{Ca}^{2+}$ ) dynamics (Zhou et al., 2013).  $\text{Ca}^{2+}$  is a versatile second messenger that regulates a variety of cellular functions ranging from cell survival, proliferation, transcriptional regulation to apoptosis (Parys and Guse, 2019; Tanwar and Motiani, 2018). It is important to note that cells can neither synthesize nor metabolize  $\text{Ca}^{2+}$  and it is the complex interplay of  $\text{Ca}^{2+}$  handling machinery ( $\text{Ca}^{2+}$  channels, pumps, ATPases

etc.) that regulates cytosolic and organellar  $\text{Ca}^{2+}$  homeostasis. In the last couple of decades, several new channels, uniporters,  $\text{Ca}^{2+}$  handling proteins etc. have been discovered. Especially, the proteins involved in a) maintaining mitochondrial  $\text{Ca}^{2+}$  dynamics b) mediating  $\text{Ca}^{2+}$  influx across plasma membrane (PM) in response to decrease in intracellular  $\text{Ca}^{2+}$  stores and c) modulating  $\text{Ca}^{2+}$  signaling in the endo-lysosomal compartments have gained significant attention in the last 10–15 years (Bagur and Hajnóczky, 2017; Parys and Guse, 2019).

Since  $\text{Ca}^{2+}$  signaling regulates plethora of cellular functions, it is not surprising that viruses hijack host cell  $\text{Ca}^{2+}$  signaling cascades for its own benefit. Indeed, a variety of viruses takeover host cell  $\text{Ca}^{2+}$  handling machinery for driving viral infection and pathogenesis. Moreover, viruses induce changes in cytosolic  $\text{Ca}^{2+}$  concentration to activate  $\text{Ca}^{2+}$  dependent enzymes for promoting their replication (Chen et al., 2019). In this review, we will provide a brief overview of the viral life cycle and cellular  $\text{Ca}^{2+}$  signaling. We will then discuss the literature demonstrating that viral proteins can interact and/or modulate activity

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of host cell  $\text{Ca}^{2+}$  handling machinery. Further, we will deliberate upon how viruses target organelle specific  $\text{Ca}^{2+}$  signaling for facilitating their entry and replication, which in turn determines viral pathogenesis. Importantly, we will prudently discuss the recent findings demonstrating the therapeutic potential of FDA-approved drugs targeting  $\text{Ca}^{2+}$  handling proteins in curtailing viral infections. Finally, we will provide future perspectives and highlight key outstanding questions in the field that demand critical and timely attention.

## 2. Viral life cycle

Viruses are microscopic obligatory intracellular parasites. Viruses live as a completely assembled virus particles known as virions (Gelderblom, 1996). The typical virion size is in the range of nanometers (Claverie, 2008; Carter, 2012). The virions are made up of two fundamental components: nucleic acid (single-stranded or double-stranded RNA or DNA) and a capsid protein coat. The capsid protects the viral genome from nucleases. Further, it helps in virion attachment to the specific receptors on the host cell during infection. The virions with an envelope are called enveloped viruses (eg. Human immunodeficiency viruses, Herpesviruses etc.), while those without an envelope are called naked viruses (eg. Papillomavirus, Parvovirus etc.).

For their replication and transmission, viruses must gain entrance into target cells and hijack the host cellular machinery. The various steps involved in the transmission of viruses inside cells are collectively referred as the “virus life cycle”. The virus life cycle can be divided into three stages i.e. entry, genome replication and exit.

### 2.1. Entry of the viral component

The viral entry into a host cell requires the identification of the viral receptor (present on host cell) by the virion. There are four stages of viral entry: attachment, penetration, cytoplasmic trafficking, and uncoating (Ryu, 2017).

#### 2.1.1. Attachment

Virion binds to specific receptors on the surface of the host cell through one or more of its surface proteins. The virion-recognition of its receptor is highly specific.

#### 2.1.2. Penetration

After attachment of the virus on host cell, the next step is penetration into the cytoplasm. The enveloped viruses use either of the following two pathways for penetrating into host cell: direct fusion or receptor-mediated endocytosis.

Direct fusion: It is a process in which two membranes (i.e., a viral envelope and a cell membrane) fuse together. The viral nucleocapsid is transmitted directly to the cytoplasm, leaving the viral envelope behind on the PM. For example: Retroviruses (Ryu, 2017).

Receptor-mediated endocytosis: Majority of the animal viruses enter the host cells through endocytosis pathway. Viruses can use different endocytosis mechanisms. The most common route is clathrin-mediated endocytosis. While some viruses utilize caveolin and lipid raft-dependent pathways (Liu, 2014).

#### 2.1.3. Intracellular trafficking

After successful penetration, the virus particles need to get to an appropriate location within the cell for genome replication. This process is called intracellular trafficking (Ryu, 2017).

#### 2.1.4. Uncoating

The uncoating is the removal of the capsid for releasing virus genome in the host cell. Depending on the virus, the uncoating can happen at one of the several sites. For example: at the cell surface, within cytoplasm, at the nuclear pore or inside the nucleus (Carter, 2012).

### 2.2. Viral replication

The replication strategy varies significantly between the different family of viruses (Liu, 2014). Regardless of the genomic composition and replication strategy, soon after infection the viral genome is expressed as functional messenger RNA (mRNA). This guides the host cell translational machinery to generate viral proteins.

### 2.3. Viral exit/egress

The viral exit from host cell can be classified into three steps: capsid assembly, release and maturation.

#### 2.3.1. Capsid assembly

The capsid assembly can be divided into two steps: capsid assembly and genome packaging. These two mechanisms may occur sequentially or concurrently, depending on the virus.

#### 2.3.2. Release

In the case of naked viruses, viral particles are released through cell lysis of the infected host cells. Therefore, post cell membrane disruption, no defined escape mechanism is required in such cases. While in the case of enveloped viruses, a mechanism in which the capsids are surrounded by a lipid bilayer takes place prior to their release (Ryu, 2017).

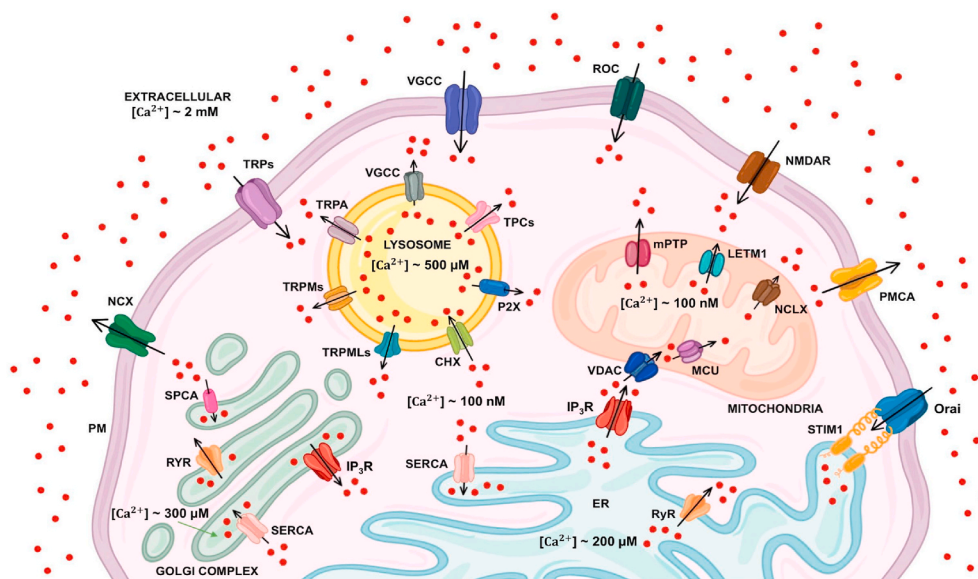
#### 2.3.3. Maturation

Maturation is the last step of the viral particle assembly that occurs extracellularly after the release of viruses from host cells. Importantly, viral particles acquire infectivity through the maturation process.

In the following sections, we will deliberate upon the molecular choreography through which viruses perturb host cell  $\text{Ca}^{2+}$  homeostasis for supporting their life cycle. First of all, we will provide a brief overview of intracellular  $\text{Ca}^{2+}$  signaling and organellar  $\text{Ca}^{2+}$  dynamics. Subsequently, we will discuss the viral targeting of  $\text{Ca}^{2+}$  homeostasis in various cellular compartments individually.

## 3. Intracellular calcium signaling

$\text{Ca}^{2+}$  is a versatile second messenger involved in regulating wide array of cellular processes including proliferation, gene transcription, aerobic metabolism, muscle contraction, exocytosis and synaptic plasticity (Parys and Guse, 2019). Under resting conditions, cells maintain cytosolic  $\text{Ca}^{2+}$  concentrations (~100 nM) via efficient coordination between the extracellular milieu ( $\text{Ca}^{2+}$  concentrations ~2 mM) and intracellular  $\text{Ca}^{2+}$  stores ( $\text{Ca}^{2+}$  concentrations ~100–200  $\mu\text{M}$ ) (Bagur and Hajnóczky, 2017; Clapham, 2007). The intracellular  $\text{Ca}^{2+}$  homeostasis is maintained by various  $\text{Ca}^{2+}$  channels and pumps present on PM and intracellular organelles such as endoplasmic reticulum (ER), mitochondria, Golgi, and lysosomes. PM has several  $\text{Ca}^{2+}$ -permeable channels and pumps such as voltage-gated calcium channels (VGCC), store-operated channels (SOC), receptor-operated channels (ROC) such as transient receptor potential channels (TRP), plasma membrane  $\text{Ca}^{2+}$  ATPase (PMCA) and the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX) (Bagur and Hajnóczky, 2017; Parys and Guse, 2019). The PM localized VGCC, SOC and ROC mediate  $\text{Ca}^{2+}$  entry from the extracellular milieu to cytosol. These PM resident channels are activated by the specific stimuli. The VGCCs are stimulated by membrane depolarization. These channels are found mostly in excitable cells (Atlas, 2014). The activation of these channels regulates various physiological functions such as excitation, contraction and relaxation of muscles, immune-protection and synaptic transmission among others (Catterall, 2011). Likewise, ROC are stimulated by external agonists, or intracellular messengers. TRP channels are major constituents of ROC and play an important role in decoding environmental stimuli. TRP channels are non-specific cationic channels that can mediate  $\text{Ca}^{2+}$  influx into the cells. The mammalian TRP channel superfamily is classified into six subfamilies based on sequence



**Fig. 1. Schematic representation of cellular  $\text{Ca}^{2+}$  handling machinery.**  $\text{Ca}^{2+}$  homeostasis is maintained by various  $\text{Ca}^{2+}$  channels and pumps present on plasma membrane (VGCC, SOC, ROC, PMCA, TRP, NCX etc.) and different intracellular compartments such as ER, mitochondria, Golgi, and lysosomes. The PM localized  $\text{Ca}^{2+}$  channels and pumps mediate  $\text{Ca}^{2+}$  entry (as shown by black arrows) from the extracellular milieu to cytosol. The  $\text{IP}_3\text{R}$  and the  $\text{RyR}$  mediate the release of  $\text{Ca}^{2+}$  from ER and Golgi.  $\text{SERCA}$  pumps back  $\text{Ca}^{2+}$  into ER and Golgi from cytosol. The  $\text{SPCA}$  mediates  $\text{Ca}^{2+}$  entry into the Golgi from cytosol.  $\text{VDAC}$ s and  $\text{MCU}$  mediate  $\text{Ca}^{2+}$  entry into the mitochondria while  $\text{NCLX}$  and  $\text{Letm1}$  facilitate  $\text{Ca}^{2+}$  extrusion from mitochondrial matrix. The  $\text{CHX}$  on lysosomes mediates  $\text{Ca}^{2+}$  entry into the lysosomes while  $\text{TPCs}$ ,  $\text{TRPMLs}$ ,  $\text{TRPMs}$  along with others facilitate  $\text{Ca}^{2+}$  extrusion from the lysosomes.  $\text{Ca}^{2+}$  ions are shown as red dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

homology: TRPC (Canonical), TRPV (Vanilloid), TRPM (Melastatin), TRPA (Ankyrin), TRPML (Mucolipin), and TRPP (Polycystic) (Samanta et al., 2018). While SOC are stimulated by the depletion of ER  $\text{Ca}^{2+}$  stores (Smyth et al., 2010). The two key players responsible for SOC channels activation are the Orai channels on the PM and ER  $\text{Ca}^{2+}$  sensor  $\text{STIM1}$  (stromal interaction molecule 1). The depletion of ER  $\text{Ca}^{2+}$  stores is sensed by  $\text{STIM1}$  leading to Orai channels opening and subsequent  $\text{Ca}^{2+}$  entry (Smyth et al., 2010; Tanwar et al., 2020). On the other hand,  $\text{PMCA}$  and  $\text{NCX}$  mediate  $\text{Ca}^{2+}$  extrusion from cytosol to extracellular space.

$\text{Ca}^{2+}$  is released from the internal stores via the  $\text{IP}_3$  receptor ( $\text{IP}_3\text{R}$ ) and the ryanodine receptors ( $\text{RyR}$ ) present on ER (Stutzmann and Mattson, 2011).  $\text{IP}_3\text{R}$  is activated by inositol-1,4,5-triphosphate ( $\text{IP}_3$ ) which leads to  $\text{Ca}^{2+}$  efflux from the stores. While the  $\text{RyR}$  releases  $\text{Ca}^{2+}$  from intracellular stores upon excitation and contraction in cardiac and skeletal muscles (Lanner et al., 2010).  $\text{Ca}^{2+}$  is pumped back into ER through sarcoplasmic/endoplasmic reticulum  $\text{Ca}^{2+}$  ATPase ( $\text{SERCA}$ ). The  $\text{Ca}^{2+}$  released from ER can be further readily taken up by mitochondria especially at sites in close proximity to the ER, known as MAMs (mitochondria associated membranes) (Csordás et al., 2006; Rizzuto et al., 1998; Sharma et al., 2020; Vecellio Reane et al., 2020). At MAMs,  $\text{Ca}^{2+}$  can easily pass through outer mitochondrial membrane to reach inter-membrane space (IMS) via non-selective voltage-dependent anion channels ( $\text{VDAC}$ s) (Madesh and Hajnóczky, 2001). From IMS,  $\text{Ca}^{2+}$  uptake into the mitochondrial matrix is mediated via a highly  $\text{Ca}^{2+}$  selective channel known as Mitochondrial  $\text{Ca}^{2+}$  Uniporter ( $\text{MCU}$ ) (Baughman et al., 2011; De Stefani et al., 2011). Mitochondrial  $\text{Ca}^{2+}$  regulates  $\text{Ca}^{2+}$ -dependent dehydrogenase enzymes, which are involved in tricarboxylic acid cycle (TCA) cycle thereby meeting cellular energy demand via ATP synthesis (Hajnóczky et al., 1995). However,  $\text{Ca}^{2+}$  overload within the mitochondrial matrix leads to apoptosis (Mammucari et al., 2018; Parks et al., 2018; Rasola and Bernardi, 2011; Tanwar et al., 2021).  $\text{Ca}^{2+}$  extrusion from mitochondrial matrix is majorly mediated through mitochondrial  $\text{Ca}^{2+}$  efflux proteins i.e. mitochondrial  $\text{Na}^+/\text{Ca}^{2+}/\text{Li}^+$  exchanger ( $\text{NCLX}$ ) (Palty et al., 2010) and leucine zipper EF hand-containing transmembrane 1 ( $\text{Letm1}$ ) (Jiang et al., 2009). Further, lysosomes can also regulate  $\text{Ca}^{2+}$  homeostasis by acting as  $\text{Ca}^{2+}$  stores (Patel and Docampo, 2010; Patel and Muallem, 2011).  $\text{Ca}^{2+}$  entry into endo-lysosomes is mediated by  $\text{Ca}^{2+}/\text{H}^+$  exchangers ( $\text{CHX}$ ) in an  $\text{H}^+$ -dependent manner (Christensen et al., 2002; Melchionda et al., 2016). While,  $\text{Ca}^{2+}$  extrusion from the endo-lysosomes is mediated

through the two-pore channel ( $\text{TPCs}$ ) (Patel, 2015; Wang et al., 2012),  $\text{TRPMLs}$  (Cheng et al., 2010),  $\text{TRPA1}$  and  $\text{TRPM2}$  (Yang et al., 2018). The intracellular cues such as nicotinic acid adenine dinucleotide phosphate ( $\text{NAADP}$ ) mobilizes  $\text{Ca}^{2+}$  from lysosomes through  $\text{TPCs}$  (Calcraft et al., 2009; Patel, 2015). In addition to ER and lysosomes, Golgi apparatus (GA) also serves as  $\text{Ca}^{2+}$  store (Dolman and Tepikin, 2006; Patel and Muallem, 2011). In membranes of GA  $\text{SERCA}$ ,  $\text{IP}_3\text{Rs}$ ,  $\text{RyRs}$  and  $\text{SPCA}$  (secretory pathway calcium ATPase) are present.  $\text{IP}_3$  binding to  $\text{IP}_3\text{R}$  releases  $\text{Ca}^{2+}$  from the Golgi.  $\text{Ca}^{2+}$  uptake in Golgi is mediated via  $\text{SERCA}$  and  $\text{SPCA}$  (Pizzo et al., 2011). The  $\text{Ca}^{2+}$  homeostasis within Golgi regulates cytosolic  $\text{Ca}^{2+}$  signaling (Raffaello et al., 2016) (Please refer Fig. 1 for the schematic illustration of the cellular  $\text{Ca}^{2+}$  handling toolkit). Importantly, dysregulation in the intracellular  $\text{Ca}^{2+}$  homeostasis is associated with several pathophysiological conditions including viral infections and pathogenesis. In the following sections, we will discuss the role of intracellular  $\text{Ca}^{2+}$  signaling in viral infections. Further, we will deliberate upon the molecular pathways adopted by viruses for hijacking host cell  $\text{Ca}^{2+}$  handling machinery to enhance viral pathogenesis.

#### 4. Viral infections and perturbations in the $\text{Ca}^{2+}$ signaling across PM

Host cell ion channels including  $\text{Ca}^{2+}$  channels are frequently targeted by viruses for their entry and replication (Charlton et al., 2020). In this section, we will discuss the molecular mechanisms through which viruses take over the PM  $\text{Ca}^{2+}$  handling machinery for their benefit.

##### 4.1. Voltage gated $\text{Ca}^{2+}$ channels (VGCC)

$\text{VGCC}$  are one of the most well studied viral targets. The availability of highly specific FDA-approved inhibitors of  $\text{VGCC}$  (e.g., verapamil and nifedipine) has further helped in evaluating their significance in viral pathogenesis. Epstein-Barr virus (EBV) causes a range of malignancies including Burkitt's lymphoma and nasopharyngeal carcinoma. EBV infection induces an increase in cytoplasmic  $\text{Ca}^{2+}$  in B lymphocytes due to augmented  $\text{Ca}^{2+}$  inflow from the extracellular space (Zhou et al., 2009). The pharmacological experiments using  $\text{VGCC}$  blockers

(verapamil or diltiazem) showed that EBV-induced human B cell activation is inhibited either upon incubation in  $\text{Ca}^{2+}$ -free medium or after treatment with VGCC blocking drugs (Dugas et al., 1989). On the contrary, no change in EBV-induced B cell activation was noted when the intracellular  $\text{Ca}^{2+}$  concentration was decreased with  $\text{Ca}^{2+}$  chelator BAPTA. This implicates a critical role for VGCC in EBV induced increase in intracellular  $\text{Ca}^{2+}$  required for B cells activation (Dugas et al., 1989).

Similarly, Nugent et al. reported that VGCC blocker Verapamil prevents influenza virus replication in canine kidney cells and in murine pulmonary macrophages (Nugent and Shanley, 1984). Recently, it was demonstrated that Influenza A virus (IAV) glycoprotein hemagglutinin (HA) binds to the Cav1.2 voltage-dependent  $\text{Ca}^{2+}$  channel (Fujioka et al., 2018). This leads to induction of intracellular  $\text{Ca}^{2+}$  oscillations and subsequent viral entry and replication. It was further reported that IAV entry is hampered by both pharmacological blocking and molecular knockdown of Cav1.2 (Fujioka et al., 2018). The small GTPase RhoA is reported to operate downstream of IAV induced  $\text{Ca}^{2+}$  response, which in turn regulates both clathrin-mediated and clathrin-independent endocytosis of virus. This signaling cascade is a key mechanism for regulating IAV internalization and infection (Fujioka et al., 2013).

HIV belongs to retroviruses family comprising of RNA as their genetic material (Karn, 2013). Two HIV-1 proteins namely the membrane glycoprotein gp120 and the transcriptional trans-activator Tat induce cytoplasmic  $\text{Ca}^{2+}$  elevation by modulating VGCC activity. These proteins have been shown to do so in both excitable (such as neuronal cells) and non-excitable cells (such as epithelial and immune cells) (Zhou et al., 2009). HIV-1 Tat interacts with L-type  $\text{Ca}^{2+}$  channels expressed by dendritic cells and inhibits cellular functions (Zocchi et al., 1998). Likewise, HIV-1 Tat targets L-type  $\text{Ca}^{2+}$  channels on NK cells, which leads to failure of NK cell-mediated cytotoxicity (Zocchi et al., 1998).

Flaviviruses include several pathogenic viruses such as Japanese encephalitis virus (JEV), Yellow Fever virus (YFV), Zika virus (ZIKV), West Nile virus (WNV), Dengue virus (DENV) etc. Excitingly, an unbiased screening of FDA-approved drugs for identifying the inhibitors of JEV infection led to identification of three VGCC blockers i.e. flunarizine, flunarizine, and flunarizine hydrochloride as highly potent blockers of JEV infection (Wang et al., 2017). In the high-throughput screening assay, these three drugs robustly inhibited the viral (wild-type JEV strain AT31) infection, which was validated by a battery of subsequent experiments (Wang et al., 2017). Since flaviviruses are quite similar in structure and pathogenesis, authors first used JEV as a model system for the drug screening and later on validated antiviral activities of VGCC blockers in ZIKV, WNV, and DENV type 2 (DENV-2) (Wang et al., 2017). Mechanistically, these drugs most likely inhibit flavivirus genome replication as they imparted complete inhibition even when cells were treated with these drugs post viral infection. This suggests that  $\text{Ca}^{2+}$  influx mediated by VGCC is critical for flavivirus genome replication.

Likewise, VGCC were identified as a therapeutic target against New World arenavirus (NWA) infection (Lavanya et al., 2013). Lavanya et al. performed a high-throughput siRNA screening with pseudo-typed virus for identifying host cell proteins of high therapeutic value. Both pharmacological blocking and siRNA mediated silencing of VGCC subunits decreased viral entry into host cells. Moreover, the authors evaluated the role of VGCC in viral infection *in vivo*. They used the vaccine strain of the Junin virus for performing *in vivo* studies in mice. Importantly, an FDA-approved VGCC targeting drug, gabapentin robustly diminished the viral infection *in vivo* thereby corroborating an important role for VGCC in NWA entry and infection (Lavanya et al., 2013). Recently, same group reported that the haploinsufficiency of one of the chains of VGCC imparts protection to human cells and mice against NWA infections (Sarute and Ross, 2020). It was demonstrated that NWA target VGCC for host cell entry and mutation in  $\alpha 1$ s chain provides significant protection against vaccine strain of the Junin virus *in vivo*. Further, the heterozygous mice carrying this mutation showed higher efficacy of gabapentin in comparison to control littermates (Sarute and Ross, 2020). Taken

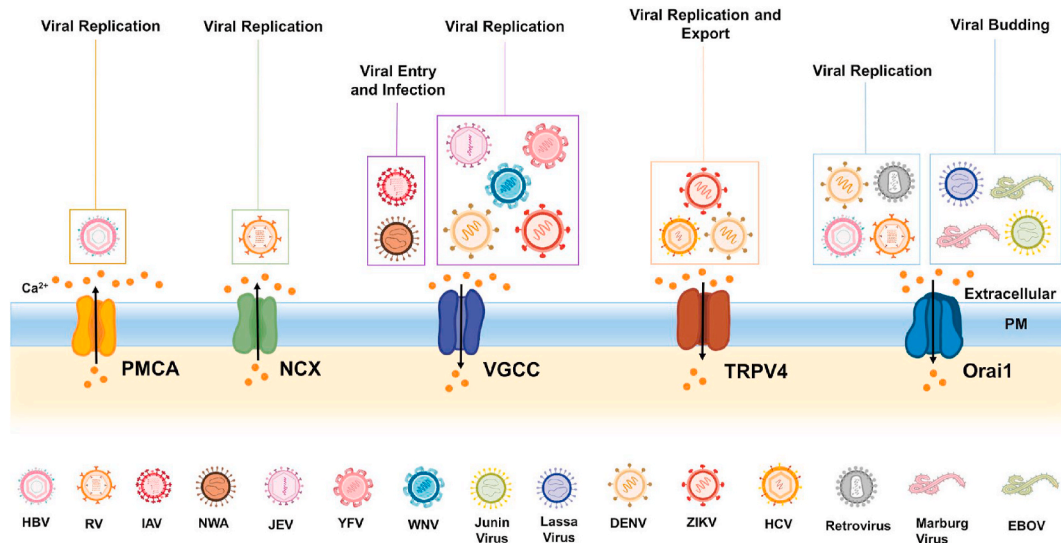
together, above discussed studies elegantly demonstrate that VGCC are attractive therapeutic targets for stalling a variety of viral infections. With a battery of FDA-approved drugs targeting VGCC, it is an area that demands immediate and critical attention for fighting against viral infections.

#### 4.2. Store operated $\text{Ca}^{2+}$ channels (SOCC)

SOCC are stimulated by the loss of  $\text{Ca}^{2+}$  from ER, which is physiologically caused by activation of a variety of cell surface receptors (Chen et al., 2019; Prakriya and Lewis, 2015). Recently, Yao et al. reported that the Hepatitis b virus (HBV) non-structural protein X (HBx) increases intracellular  $\text{Ca}^{2+}$  by interacting with SOCC protein Orai1 (Yao et al., 2018). The authors performed confocal microscopy, co-immunoprecipitation and GST-pull-down assay for demonstrating that Orai1 C-terminus interacts with HBx (Yao et al., 2018). This interaction enhances store operated  $\text{Ca}^{2+}$  entry, thereby elevating cytosolic  $\text{Ca}^{2+}$  levels. It is important to note that HBx mediated modulation of intracellular  $\text{Ca}^{2+}$  dynamics is critical for HBV replication. Further, HBx regulates viral pathogenesis, host cell apoptosis, transcription and cell signaling (Bouchard and Navas-Martin, 2011). Consequences of HBx expression vary according to the cell type (Bouchard and Navas-Martin, 2011). Recently, a direct association between HBx mediated cytosolic  $\text{Ca}^{2+}$  elevation (via SOCC) and stimulation of HBV replication was reported (Casciano et al., 2017). It was demonstrated that expression and activity of SOCC components remain unaltered but  $\text{Ca}^{2+}$  entry via SOCC is prolonged. The persistent high cytosolic  $\text{Ca}^{2+}$  levels stimulate downstream effector proteins, which in turn creates favorable cellular environment for HBV replication.

In case of retroviruses, pX open reading frame I encoding two proteins i.e. p12I and p27I is necessary for developing persistent infection (Ding et al., 2002). The chemical inhibition experiments using SOCC inhibitor indicated that SOCC contribute to the p12I-mediated Nuclear Factor of Activated T-cells (NFAT) activation (Ding et al., 2002). The NFAT activation in turn leads to the replication of retroviruses (Ding et al., 2002). Similarly, rotavirus (RV) infection enhances cytosolic  $\text{Ca}^{2+}$  concentration, which is critical for virus replication (Chang-Graham et al., 2019). The pharmacological experiments demonstrated that both RV infection and RV nonstructural protein 4 (NSP4) induce a rise in cytoplasmic  $\text{Ca}^{2+}$  levels via SOCC (Díaz et al., 2012). Subsequently, Hyser et al. reported that in RV infected cells, STIM1 is constitutively active and STIM1 puncta are colocalized with the Orai1 (Hyser et al., 2013). The wild-type NSP4 was shown to activate STIM1, which results in  $\text{Ca}^{2+}$  influx. But an NSP4 viroporin mutant failed to cause STIM1 oligomerization and did not activate the SOCC entry (SOCE) (Hyser et al., 2013). Importantly, STIM1 knockdown substantially decreased RV yield, suggesting that STIM1 plays a vital role in RV replication (Hyser et al., 2013). Recently, it was demonstrated that the inhibition of SOCE or Orai1 channel attenuates the cytosolic  $\text{Ca}^{2+}$  spikes critical for viral replication (Chang-Graham et al., 2019). Taken together, these studies establish an important role of SOCC mediated increase in cytosolic  $\text{Ca}^{2+}$  in RV replication.

Likewise, it was recently demonstrated that DENV infection in human hepatic cells leads to activation of SOCC channels and rise in cytosolic  $\text{Ca}^{2+}$  levels via Orai1 (Dionicio et al., 2018). It was observed that DENV infection induces ER  $\text{Ca}^{2+}$  store depletion followed by co-localization of STIM1 and Orai1 leading to  $\text{Ca}^{2+}$  influx via Orai1. Further, the treatment of infected cells with  $\text{Ca}^{2+}$  chelators (BAPTA-AM and EGTA) or SOCC inhibitor (SKF96365) substantially reduced the viral titers, thereby emphasizing (Dionicio et al., 2018) that SOCC mediated  $\text{Ca}^{2+}$  influx plays an important role in DENV replication. Similarly, Han et al. demonstrated that hemorrhagic fever viruses like the filoviruses (Ebola and Marburg) and arenaviruses (Lassa and Junín viruses) induce rise in cytosolic  $\text{Ca}^{2+}$  levels via STIM1/Orai1-mediated SOCE (Han et al., 2015). The authors silenced STIM1/Orai1 as well as pharmacologically blocked SOCE for demonstrating a critical role of this pathway in



**Fig. 2. PM  $\text{Ca}^{2+}$  handling toolkit and viral infections.** A variety of viruses target PM  $\text{Ca}^{2+}$  handling machinery for increasing cytosolic  $\text{Ca}^{2+}$  levels and that in turn typically helps in driving viral replication and export. Viruses like JEV, DENV, ZIKV, YFV, WNV target VGCC for driving viral replication. Similarly, RV, HIV, HBV stimulate Orai1 mediated  $\text{Ca}^{2+}$  influx for enhancing replication. Whereas, ZIKV, HCV and DENV activate TRPV4 dependent signaling cascade for potentiating their replication and exit from host cells. Viruses also target PM  $\text{Ca}^{2+}$  efflux routes such as PMCA and NCX. For example, HBV and RV target PMCA and NCX respectively, for elevating cytosolic  $\text{Ca}^{2+}$  levels and this in turn aids in viral replication.

enhancing cellular  $\text{Ca}^{2+}$  levels upon filo and arenavirus infection. Importantly, STIM1 and Orai1 regulate viral budding and pathogenesis of Ebola, Marburg, Lassa and Junin viruses (Han et al., 2015) thereby suggesting that SOCE could be targeted for controlling the pathogenesis of hemorrhagic fever viruses. In summary, STIM1-Orai1 mediated SOCE plays a critical role in viral replication and budding in a battery of different viral families.

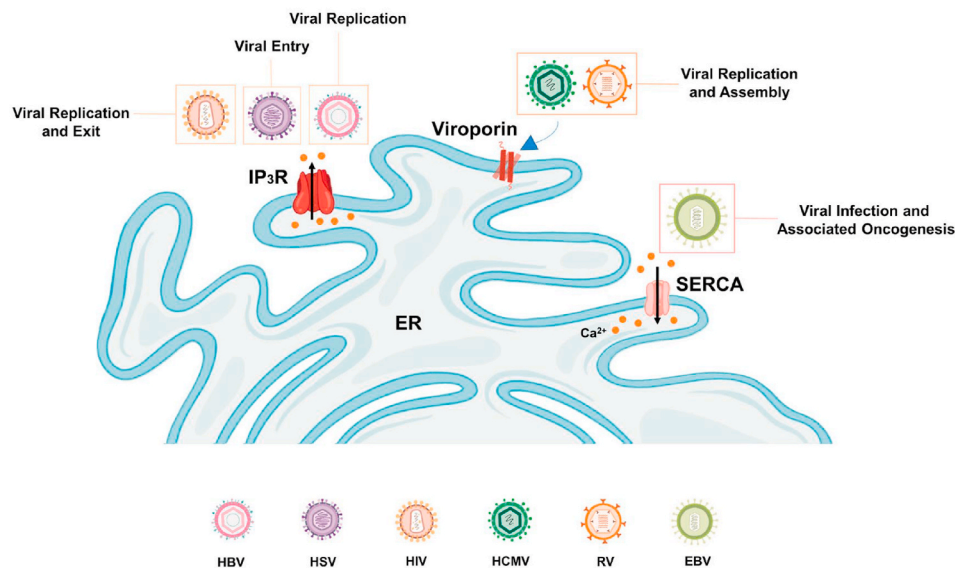
#### 4.3. Receptor operated $\text{Ca}^{2+}$ channels (ROCC)

The role of ROCC in viral infections remains poorly understood. The infection of HIV-1 coat protein gp120 in rat retinal ganglion cells and hippocampal neurons induces significant increase in the intracellular  $\text{Ca}^{2+}$  leading to cytotoxicity (Nath et al., 1995). In gp120-mediated biological responses, glutamate is involved (Nath et al., 1995). The gp120 activity is blocked, at least in part, by NMDA-selective excitatory amino acid receptor antagonists,  $\text{Ca}^{2+}$  channel inhibitors (like dantrolene) and nitric oxide synthase inhibitors (Nath et al., 1995). Recent studies have reported that the two Tat protein variants, Tat 1–72 and Tat 1–86 induce rapid rise in intracellular  $\text{Ca}^{2+}$  in rat hippocampus organotypic slice cultures (Self et al., 2004). Importantly, NMDA receptor antagonist dizocilpine significantly attenuated changes in intracellular  $\text{Ca}^{2+}$  levels induced by Tat 1–72 or Tat 1–86. This suggests that targeting NMDA receptor could help in managing neurotoxic effects associated with HIV infection (Self et al., 2004). More recently, transient receptor potential vanilloid 4 (TRPV4) was reported to regulate replication of a variety of viruses. It was demonstrated that TRPV4 is frequently expressed after viral infection, exhibits some spontaneous activity, and induces intracellular  $\text{Ca}^{2+}$  signals (Doñate-Macián et al., 2018). TRPV4 binds and controls the functioning of the DEAD-box RNA helicase DDX3X. DDX3X is attacked by many RNA viruses. TRPV4-mediated  $\text{Ca}^{2+}$  influx leads to release of DDX3X from the channel and its subsequent nuclear translocation (Doñate-Macián et al., 2018). The molecular knockdown or pharmacological inhibition of TRPV4 reduces DDX3X-dependent functions such as translation and export of viral proteins (Doñate-Macián et al., 2018). Additionally, TRPV4 mediates nuclear aggregation of DDX3X in cells infected with Zika virus. Importantly, TRPV4 targeting decreases dengue, hepatitis C and Zika virus infectivity (Doñate-Macián et al., 2018).

#### 4.4. Plasma membrane $\text{Ca}^{2+}$ ATPase (PMCA) and sodium-calcium exchanger (NCX)

As major  $\text{Ca}^{2+}$  extruders, PMCA and NCX take  $\text{Ca}^{2+}$  out of the cell, thereby helping in maintaining cytosolic  $\text{Ca}^{2+}$  homeostasis. Viruses can directly or indirectly regulate the activity of PMCA and/or NCX for inducing changes in the cytosolic  $\text{Ca}^{2+}$  levels. For example, the ectopic expression of the HBx protein causes elevation of the cytosolic  $\text{Ca}^{2+}$  by modulating PMCA activity (Chami et al., 2003; Zhou et al., 2013). HBV infection did not modify the levels of PMCA-1, 2, 3, or 4 mRNAs suggesting that HBV does not influence PMCA expression at least at the mRNA level (Casciano et al., 2017). Mechanistically, it was demonstrated that HBx overexpression induces caspase-3-mediated cleavage of PMCA while blockage of caspase activity abolishes PMCA cleavage (Chami et al., 2003). Therefore, HBx regulates PMCA activity by inducing its cleavage. This in turn decreases  $\text{Ca}^{2+}$  efflux from the cytosol to the extracellular environment resulting in elevated cytoplasmic  $\text{Ca}^{2+}$  levels (Casciano et al., 2017). The rise in cytosolic  $\text{Ca}^{2+}$  concentration augments HBV replication and enhances the core assembly of HBV (Zhou et al., 2013). Similarly, RV infection dysregulates cellular  $\text{Ca}^{2+}$  homeostasis, which in turn helps in its replication and diarrheal pathogenesis (Chang-Graham et al., 2020). RV NSP4 is responsible for inducing perturbations in host cell  $\text{Ca}^{2+}$  dynamics (Sastri et al., 2016). Diaz et al. reported that RV infection or just NSP4 expression leads to reverse mode activation of NCX thereby increasing the cytosolic  $\text{Ca}^{2+}$  concentration. This suggests that RV targets NCX and stimulates its functioning in reverse mode, which in turn results in rise in cellular  $\text{Ca}^{2+}$  levels required for RV replication (Díaz et al., 2012).

Taken together, above studies demonstrate that a variety of viruses hijack different types of PM  $\text{Ca}^{2+}$  handling proteins for the host cell entry and replication (please refer Fig. 2 for the diagrammatic illustration). Most of these viruses enhance cytosolic  $\text{Ca}^{2+}$  concentration for driving their replication. However, the molecular choreography connecting rise in cytosolic  $\text{Ca}^{2+}$  levels to augmented viral replication remains poorly understood. Certainly, it is critical to enrich this knowledge base so that it could be utilized in the future for developing therapeutic strategies in curtailing the replication of such viruses.



**Fig. 3. ER Ca<sup>2+</sup> homeostasis and viral infections.** A number of viruses target ER Ca<sup>2+</sup> handling toolkit, which in turn aids in all three stages of infection i.e. viral entry (eg. HSV), viral replication (eg. HIV and HBV) and viral exit (eg. HIV). Some viruses, such as RV and HCMV can induce ER Ca<sup>2+</sup> efflux by forming viroporin in the ER membrane. This in turn helps in viral replication and assembly. While oncogenic EBV targets SERCA regulated ER Ca<sup>2+</sup> homeostasis for driving viral infection and associated oncogenesis.

## 5. ER Ca<sup>2+</sup> homeostasis and viral infections

Viruses exploit ER to promote various steps of their life cycle (Romero-Brey and Bartenschlager, 2016). A variety of viruses target host cell ER Ca<sup>2+</sup> stores for driving their replication and enhancing viral infection. For example RV infection leads to elevation of cytosolic Ca<sup>2+</sup> levels in the host cell because of increased efflux of Ca<sup>2+</sup> from ER into cytosol followed by Ca<sup>2+</sup> influx through PM (Hyser and Estes, 2015; Pérez et al., 1999; Tian et al., 1994). The virus encoded NSP4 protein has been reported to be responsible for the changes in Ca<sup>2+</sup> dynamics (Hyser and Estes, 2015; Tian et al., 1994). NSP4 perturbs Ca<sup>2+</sup> homeostasis by at least two mechanisms: viroporin function and endotoxin activity (Sastri et al., 2016). NSP4 is localized in the ER and functions as a viroporin that depletes ER Ca<sup>2+</sup> stores. This leads to rise in cytosolic Ca<sup>2+</sup> levels, which is important for replication of RV (Díaz et al., 2008; Hyser et al., 2010, 2013; Pham et al., 2017; Tian et al., 1995). Secondly, NSP4 possesses an enterotoxin domain (amino acids 114–135), which can induce chloride secretion from enterocytes due to PLC-dependent transient rise in cytosolic Ca<sup>2+</sup> (Dong et al., 1997; Morris et al., 1999). Recently, Chang-Graham et al. characterized RV modulated Ca<sup>2+</sup> signaling by stably expressing genetically encoded Ca<sup>2+</sup> indicators targeting cytosol and/or ER in cell lines and human intestinal enteroids (HIEs) (Chang-Graham et al., 2019). The virus induced increase in cytosolic Ca<sup>2+</sup> exhibits spatial and temporal complexity. The authors measured alterations in cytosolic Ca<sup>2+</sup> in single cells over a long time to understand if RV dysregulates Ca<sup>2+</sup> levels during infection and its progression. They observed that release of Ca<sup>2+</sup> from the ER is the prime cause of RV-induced cytosolic Ca<sup>2+</sup> spikes. Importantly, modulation of Ca<sup>2+</sup> signals by RV can direct the host cell either towards cell death or cell survival/proliferation. RV infection activates discrete, transient Ca<sup>2+</sup> signaling events rather than a steady rise in cytosolic Ca<sup>2+</sup> levels (Chang-Graham et al., 2019). This acts as a pro-survival signal by stimulating PI3K and calcineurin-dependent NFAT transcription factor. As Ca<sup>2+</sup> levels increase drastically during the later stages of viral life cycle, cell damage and death occur (Crawford et al., 2012; Pérez et al., 1998; Zhu et al., 2017). In summary, RV targets ER Ca<sup>2+</sup> signaling for promoting its replication and assembly.

Human cytomegalovirus (HCMV) encodes several genes (US21, UL37, US28) that can regulate host cell Ca<sup>2+</sup> signaling (Dunn and Munger, 2020). US21 gene encodes a Ca<sup>2+</sup> permeable channel, a viroporin, that depletes the ER Ca<sup>2+</sup> stores (Luganini et al., 2018). This in turn decreases susceptibility of the cell to apoptosis. Likewise, UL37 is responsible for mobilizing Ca<sup>2+</sup> from ER to cytosol thereby enhancing

ATP production, which is used to meet energy demands of the virus (Sharon-Friling et al., 2006). On the contrary, US28 (a viral GPCR) activates caspase that promotes apoptosis (Gao and Murphy, 1994; Pleskoff et al., 2005). This aids in disease progression in certain specific cell types. Miller et al. reported that US28 triggers PLC-β signaling which leads to release of intracellular Ca<sup>2+</sup> in smooth muscle and endothelial cells but not in glioblastoma cells (Miller et al., 2012). Further, UL146 encodes vCXCL1 (a viral chemokine), which is an agonist for human chemokine receptors (CXCR1 and CXCR2) and it induces intracellular Ca<sup>2+</sup> release (Penfold et al., 1999; Wang et al., 2003). Additionally, interaction between HCMV and epidermal growth factor receptor (EGFR) is responsible for internalization of the virus. As a consequence, intracellular Ca<sup>2+</sup> levels are increased (Wang et al., 2003) and that aids in viral dissemination through the host. In summary, HCMV gene products modulate ER Ca<sup>2+</sup> signaling via multiple cascades eventually regulating host cell growth, apoptosis and inflammatory responses.

EBV, an oncogenic gamma-herpes virus infects normal resting B lymphocytes and leads to immortalization of the cells (Papp et al., 2020). EBV has the ability to immortalize primary naive B lymphocytes, which results in various forms of lymphomas (Dellis et al., 2009). EBV infection leads to a rise in resting cytosolic Ca<sup>2+</sup> levels (Chami et al., 2006; Scharenberg et al., 2007) by modulating the expression and activity of SERCA3 (Dellis et al., 2009). EBV protein, LMP-1 (latent membrane protein-1) brings about the down-regulation of SERCA3. This process eventually results in B cell immortalization associated with EBV infection.

Ca<sup>2+</sup> signaling plays an important role in HIV-1 protein trafficking, release and viral replication. HIV-1 gp120 and Tat are responsible for IP<sub>3</sub> generation (Ehrlich et al., 2011, 2014) whereas HIV Nef acts as an agonist for IP<sub>3</sub>R (Ehrlich et al., 2010; Manninen and Saksela, 2002). The activation of IP<sub>3</sub>R is required for efficient trafficking of HIV-1 Gag and for viral release (Ehrlich et al., 2010). Furthermore, the studies from Ehrlich et al. indicate that Ca<sup>2+</sup> is required for Gag assembly, which is facilitated by IP<sub>3</sub>R mediated ER Ca<sup>2+</sup> release. The authors demonstrated that Gag regulates both ER Ca<sup>2+</sup> release and store refilling (Ehrlich et al., 2014). Interestingly, Gag stimulates IP<sub>3</sub>R and distributes them towards the cell periphery (Chalmers et al., 2006; Vermassen et al., 2004). Further, it is reported that functioning of PI(4,5)P<sub>2</sub> as a PM anchor and PLC substrate are required for efficient HIV infection. Some of the ER released Ca<sup>2+</sup> might keep activating PLC, which hydrolyses PI(4,5)P<sub>2</sub> and provide a constant supply of IP<sub>3</sub> and hence support continuous activation of IP<sub>3</sub>R (Rhee, 2001; Suh et al., 2008). Importantly, the increase in production of viral particles is observed upon ER Ca<sup>2+</sup> release

(Ehrlich et al., 2014). Similarly, HIV protein Nef plays an important role in AIDS pathogenesis (Manninen and Saksela, 2002). It acts as an agonist for IP<sub>3</sub>R and activates it directly (Ehrlich et al., 2010; Manninen and Saksela, 2002). This in turn induces ER Ca<sup>2+</sup> release and enhances HIV replication (Manninen and Saksela, 2002).

The accumulation of HBV protein variants HBsAg and HBeAg in the ER (Hsieh et al., 2004; Lee et al., 2015a, 2015b) leads to release of ER Ca<sup>2+</sup> into the cytosol and increases ROS production (Lee et al., 2015a). Moreover, ER Ca<sup>2+</sup> stores are released upon activation by HCV (Wang et al., 2010). The released Ca<sup>2+</sup> subsequently accumulates in the mitochondria and enhances ROS generation (Li et al., 2007; Piccoli et al., 2007; Sharma et al., 2020). The ability of HCV and HBV to deplete ER Ca<sup>2+</sup> stores leads to hepato-carcinogenesis induction (Bartosch et al., 2009; Ivanov et al., 2017). Cheshenko et al. demonstrated that upon Herpes simplex virus (HSV) interaction with host cells, phosphoinositide-specific PLC $\gamma$  is activated (Cheshenko et al., 2003). This triggers ER Ca<sup>2+</sup> release downstream of PLC $\gamma$ -IP<sub>3</sub> pathway. It leads to activation of phosphorylation pathways, which aids in viral penetration and delivery of viral capsids to host cell nucleus. In a subsequent study, same group used confocal microscopy to demonstrate that viral penetration triggers rise in cellular Ca<sup>2+</sup> via release of IP<sub>3</sub> sensitive intracellular Ca<sup>2+</sup> stores. Importantly, the abrogation of intracellular Ca<sup>2+</sup> response prevented viral entry and infection. The inhibition of IP<sub>3</sub>R and chelation of intracellular Ca<sup>2+</sup> inhibits viral infection. This suggests that IP<sub>3</sub> induced Ca<sup>2+</sup> release from intracellular stores plays an important role in regulating HSV entry in the host cells (Cheshenko et al., 2007).

Taken together, above studies clearly exhibit that viral proteins target ER Ca<sup>2+</sup> handling machinery for modulating ER and cytosolic Ca<sup>2+</sup> levels. This in turn helps in viral entry, replication and exit thereby playing an important role in viral pathogenesis (Refer Fig. 3 for the pictorial summary). However, more mechanistic investigations are required to precisely decipher how changes in ER Ca<sup>2+</sup> drive viral infections.

## 6. Mitochondrial Ca<sup>2+</sup> dynamics and viral infections

Mitochondrial Ca<sup>2+</sup> homeostasis is a critical regulator of host cell fate upon viral infection (Zhou et al., 2009). Mitochondria are the key targets for viruses as they are major player involved in regulating bioenergetics and apoptosis (Cavallari et al., 2018). Several studies have demonstrated that host cell mitochondrial Ca<sup>2+</sup> signaling is altered during viral infections (Anand and Tikoo, 2013; Chaudhuri et al., 2021). Viruses modulate the Ca<sup>2+</sup> flux across mitochondria to induce or prevent apoptosis (Panda et al., 2021). Host cells utilize apoptosis as an innate defense mechanism to control virus production and viral dissemination (Benedict et al., 2002). In contrast, viruses utilize an anti-apoptotic approach to evade host immune clearance. Interestingly, certain viruses induce apoptosis to aid viral dissemination (Zhou et al., 2009). Various types of viruses induce perturbations in mitochondrial Ca<sup>2+</sup> signaling to aid persistent infection (Anand and Tikoo, 2013; Chaudhuri et al., 2021; Ohta and Nishiyama, 2011).

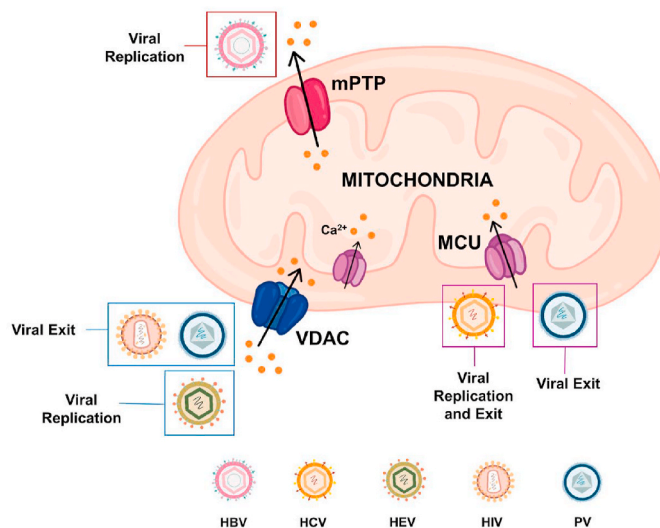
The HBx protein plays a critical role in replication of HBV and the development of liver cancer (Bouchard and Schneider, 2004). Several independent studies have reported localization of HBx to mitochondria (Clippinger and Bouchard, 2008; Henkler et al., 2001; Huh and Siddiqui, 2002; Takada et al., 1999). By utilizing aequorin-based recombinant probes, it was revealed that overexpression of HBx in HepG2 and HeLa cells leads to decrease in histamine induced mitochondrial Ca<sup>2+</sup> uptake (Chami et al., 2003). Furthermore, the ectopic expression of HBx altered mitochondrial morphology i.e. round, fragmented and swollen mitochondria (Chami et al., 2003). Taken together, this study suggested that HBx induces apoptosis by modulating mitochondrial Ca<sup>2+</sup> signaling. Several groups have reported a critical role for mitochondrial Permeability Transition Pore (mPTP) activity in HBV replication (Bouchard et al., 2001; Gearhart and Bouchard, 2010; McClain et al., 2007). An interesting report by Gearhart et al. implicated a role for mitochondrial

Ca<sup>2+</sup> signaling in replication of HBV (Gearhart and Bouchard, 2010). The authors demonstrated that HBx causes cell cycle arrest in G<sub>1</sub> phase via mitochondria-dependent Ca<sup>2+</sup> signaling. This in turn leads to stimulation of HBV replication (Gearhart and Bouchard, 2010). It was reported that mitochondrial Ca<sup>2+</sup> dynamics regulates HBx activated HBV DNA replication (Bouchard et al., 2001). The treatment with cyclosporin A (CsA), a mPTP inhibitor reduces HBx activated HBV DNA replication (Bouchard et al., 2001). In an interesting study, Rahmani et al. described interaction between HBx and VDAC3 within mitochondrial membrane (Rahmani et al., 2000). The authors further reported altered mitochondrial membrane potential upon HBx expression in human hepatoma cells (Rahmani et al., 2000). Similarly, Yang et al. reported that HBx protein induces rise in cytosolic Ca<sup>2+</sup> by modulating mitochondrial Ca<sup>2+</sup> uptake (Yang and Bouchard, 2012). The authors demonstrated an increase in mitochondrial Ca<sup>2+</sup> uptake in HBx-expressing cells. This increased mitochondrial Ca<sup>2+</sup> uptake stimulates HBV replication (Yang and Bouchard, 2012).

The HCV infection is associated with various mitochondrial dysfunctions (Brault et al., 2013; Piccoli et al., 2006). Piccoli et al. revealed that expression of HCV protein leads to dysregulation of mitochondrial Ca<sup>2+</sup> homeostasis in U2-OS human osteosarcoma cells (Piccoli et al., 2007). The rise in mitochondrial Ca<sup>2+</sup> influx led to mitochondrial Ca<sup>2+</sup> overload, which in turn results in altered bioenergetics, inhibition of complex I, decreased mitochondrial membrane potential, increased ROS and elevated nitro-oxidative stress. Importantly, these observations could be completely reversed upon inhibition of mitochondrial Ca<sup>2+</sup> uptake by ruthenium red or Ru360 (Piccoli et al., 2007). Similarly, Gong et al. demonstrated the role of HCV nonstructural protein 5 A (NS5A) in altering mitochondrial Ca<sup>2+</sup> homeostasis and inducing nuclear translocation of NF- $\kappa$ B and STAT-3 transcription factors (Gong et al., 2001). A decrease in NS5A-induced NF- $\kappa$ B activation was observed upon inhibition of mitochondrial Ca<sup>2+</sup> uptake by ruthenium red (RR). Thus, suggesting a critical role for mitochondrial Ca<sup>2+</sup> in regulating NS5A's ability to activate transcription factors (Gong et al., 2001). Likewise, Li et al. reported that HCV core protein enhances mitochondrial Ca<sup>2+</sup> uptake and ROS generation by potentiating the activity of Ca<sup>2+</sup> uniporter (Li et al., 2007). Further, the authors observed a rise in mitochondrial Ca<sup>2+</sup> influx upon incubating mouse liver mitochondria with HCV core protein *in vitro*. This increased entry was inhibited by the mitochondrial Ca<sup>2+</sup> uniporter inhibitor Ru360 but not with Na<sup>+</sup>/Ca<sup>2+</sup> exchanger inhibitor or ROS scavengers. This suggests that the mitochondrial Ca<sup>2+</sup> uniporter is one of the targets of HCV infection (Li et al., 2007). Interestingly, Quarato et al. reported that alisporivir (an analogue of mPTP inhibitor cyclosporine A and cyclophilin inhibitor) treatment gives protection against HCV-induced mitochondrial dysfunction (Quarato et al., 2012). The authors further demonstrated that alisporivir rescues HCV-induced increase in ROS production, depolarization of mitochondrial membrane potential and mitochondrial Ca<sup>2+</sup> overload. Therefore, this study suggests a potential therapeutical value of alisporivir in managing HCV infections (Quarato et al., 2012).

A study by Moin et al. reported that hepatitis E virus open reading frame 3 (Orf3) protein provides protection to virus from mitochondrial depolarization and associated cell death (Moin et al., 2007). The authors reported that stable Orf3-expression leads to upregulation of VDAC. Further, siRNA mediated knockdown of Orf3 decreases VDAC expression. The authors showed a decrease in cell death in Orf3-expressing cells. This was associated with the reduction in OMM permeabilization via VDAC1 oligomerization and increase in Orf3 interaction with hexokinase I. This signaling cascade eventually prevents the release of cytochrome c and aids in cell survival, thereby allowing viral replication in these cells (Moin et al., 2007). Similarly, HCMV UL37 encodes a viral mitochondria-localized inhibitor of apoptosis (vMIA), which promotes cell survival by inhibiting a pro-apoptotic protein Bax (Arnoult et al., 2004; Goldmacher et al., 1999; Poncet et al., 2004). All the cited studies collectively recognize UL37 as a potent inhibitor of host cell apoptosis that in turn helps in continuous viral replication.





**Fig. 4. Mitochondrial  $\text{Ca}^{2+}$  dynamics and viral infections.** Viruses act on mitochondrial  $\text{Ca}^{2+}$  dynamics for driving their replication and for inducing host cell apoptosis which in turn helps in viral exit. For example, HBV, HCV and HEV target mPTP, MCU and VDAC respectively for enhancing their replication. While HIV and PV stimulate VDAC and MCU activity thereby increasing mitochondrial  $\text{Ca}^{2+}$  concentration. The rise in mitochondrial  $\text{Ca}^{2+}$  levels leads to host cell apoptosis.

HIV viral proteins (Tat and gp120) induce mitochondrial impairment by perturbing processes such as mitochondrial dynamics, morphology and mitochondrial membrane potential (Rozzi et al., 2017). Kruman et al. reported that Tat induces apoptosis of cultured embryonic rat hippocampal neurons via caspase activation and mitochondrial  $\text{Ca}^{2+}$  overloading (Kruman II et al., 1998). The authors demonstrated that Tat induces rise in cytosolic  $\text{Ca}^{2+}$  leading to increase in mitochondrial  $\text{Ca}^{2+}$  uptake and generation of mitochondrial ROS. Furthermore, they showed protection against Tat-induced apoptosis with BAPTA-AM and ruthenium red (Kruman II et al., 1998) suggesting an important role for mitochondrial  $\text{Ca}^{2+}$  homeostasis in regulating host cell survival post HIV infection. Subsequent studies reported that Tat protein targets VDAC (Lecoer et al., 2012). Lecoer et al. demonstrated that Tat protein induces mitochondrial membrane permeabilization and inhibition of cytochrome *c* oxidase (COX) activity (Lecoer et al., 2012). Importantly, it was shown that VDAC inhibition with 4,4'-diisothiocyanostilbene-2, 2'-disulfonic acid (DIDS) leads to protection against Tat-induced mitochondrial membrane permeabilization (Lecoer et al., 2012). Likewise, Brisac et al. demonstrated an important role for mitochondrial  $\text{Ca}^{2+}$  in poliovirus (PV) induced apoptosis of neuroblastoma cells (Brisac et al., 2010). The authors showed that PV infection leads to increase in cytosolic  $\text{Ca}^{2+}$ . This in turn leads to rise in mitochondrial  $\text{Ca}^{2+}$  via VDACS and MCU. This increase in mitochondrial matrix  $\text{Ca}^{2+}$  concentration results in mitochondrial dysfunction and apoptosis in PV-infected cells (Brisac et al., 2010). It was further demonstrated that pretreatment of cells with ruthenium red or DIDS leads to reduction in cytochrome *c* release and PV-induced apoptosis (Brisac et al., 2010). Taken together, these studies suggest that HIV and PV target VDAC and MCU for inducing host cell apoptosis.

The group B coxsackieviruses (CVB) are ssRNA viruses that cause meningitis and myocarditis. The main pathogen leading to viral myocarditis is Coxsackievirus B3 (CVB3) (Wessler and Backert, 2011). Emerging data indicates that replication of CVB3 is correlated with apoptosis of the infected cardiac myocytes (Nie et al., 2020). In the CVB3-infected H9c2 cardiomyocytes,  $\text{Ca}^{2+}$  calmodulin-dependent kinase II (CaMKII) was activated by intracellular  $\text{Ca}^{2+}$  overload (Nie et al., 2020). Importantly, CVB3-induced CaMKII activation was reported to enhance mitochondrial  $\text{Ca}^{2+}$  uptake, activating

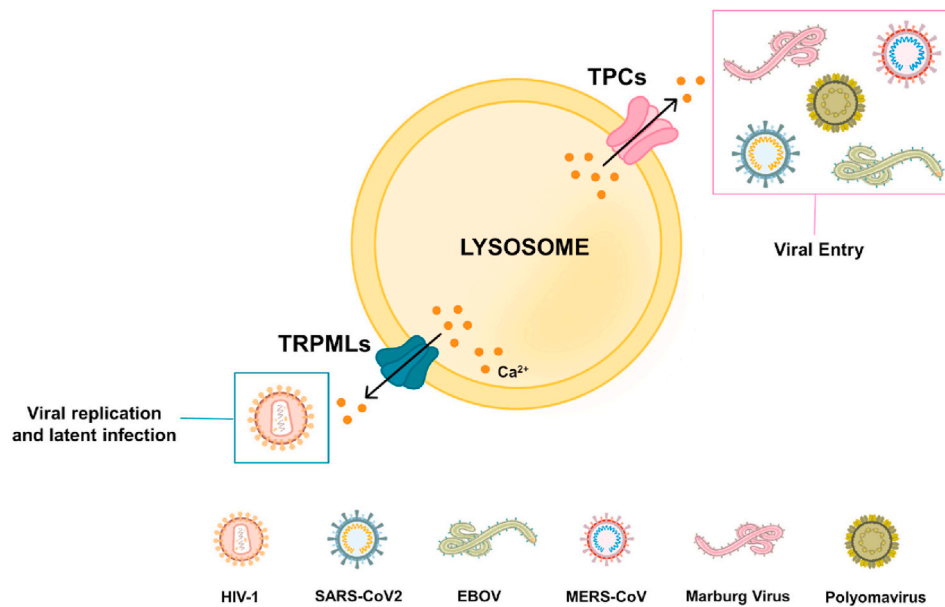
mitochondrial-dependent apoptotic pathways. Further, CaMKII inhibition with a pharmacological inhibitor (KN-93) or its silencing by shRNA repressed cytochrome *c* release from mitochondria and abrogated CVB3-induced H9c2 apoptosis. While overexpression of the CaMKII activated mutant (CaMKII-T287D) increased CVB3-induced H9c2 apoptosis (Nie et al., 2020) thereby suggesting that mitochondrial  $\text{Ca}^{2+}$  dynamics play a critical role in CVB3 pathogenesis.

Collectively, above discussed studies establish that a number of different viruses perturb mitochondrial  $\text{Ca}^{2+}$  signaling either for enhancing their replication or for inducing host cell apoptosis which in turn aids in viral exit (Please refer to Fig. 4 for the diagrammatic summary). Recently, a high throughput screening of small molecules including NIH clinical collection drug library led to identification of highly specific drugs that can modulate mitochondrial  $\text{Ca}^{2+}$  dynamics (Arduino et al., 2017; Di Marco et al., 2020). Going forward, it would be interesting to evaluate the efficacy of these drugs in controlling viral infections associated with dysregulation of mitochondrial  $\text{Ca}^{2+}$  homeostasis.

## 7. Lysosomal $\text{Ca}^{2+}$ dynamics and viral entry

The lysosomal  $\text{Ca}^{2+}$  drives important cellular processes such as signal transduction, vesicular trafficking, autophagy, exocytosis etc. (Feng and Yang, 2016; Lloyd-Evans and Platt, 2011; Patel and Cai, 2015; Trivedi et al., 2020). Viruses target lysosomal  $\text{Ca}^{2+}$  handling machinery for entry into host cells. A fascinating study by Sakurai et al. reported an important role for endo-lysosomal TPCs in EBOV entry into host cells (Sakurai et al., 2015). The authors demonstrated that Ned19 (TPC inhibitor) and tetrandrine (NAADP antagonist) treatment reduces Ebola virus (EBOV) infection. A robust decrease in EBOV infection was observed in the mouse embryonic fibroblasts harvested from TPC1/2 knockout mice thereby confirming an essential role for TPCs in EBOV entry. Furthermore, tetrandrine treatment led to inhibition of Marburgvirus (distantly related filovirus) signifying a critical role of TPCs in filovirus infection (Sakurai et al., 2015). Independently, Simmons et al. reported that EBOV entry occurs through endolysosomes positive for two-pore  $\text{Ca}^{2+}$  channel 2 (TPC2) and Niemann-Pick C1 (NPC1) (Simmons et al., 2016). A recent study by Penny et al. identified FDA-approved blockers for TPC to inhibit viral entry into host cells (Penny et al., 2019). For identifying novel compounds targeting TPC, the authors virtually screened a library of around 1500 FDA-approved drugs by performing TPC2 structure-based virtual drug screening. It was observed that the drugs targeting dopamine and estrogen receptors bind to TPC pore. Further *in vitro* validation studies demonstrated that these drugs indeed inhibit endogenous NAADP-evoked  $\text{Ca}^{2+}$  release and TPC2 channel activity. Most importantly, these drugs led to a significant reduction in EBOV infection in HeLa cells. Taken together, these elegant studies established an important role for TPCs in EBOV entry and identified TPCs as a potential drug target against EBOV infection (Penny et al., 2019). More recently, Das et al. reported a role for  $\text{Ca}^{2+}$  in EBOV entry by utilizing single-molecule FRET (smFRET)-imaging (Das et al., 2020). These authors demonstrated that pH,  $\text{Ca}^{2+}$ , and NPC1 receptor binding leads to conformational changes in EBOV envelope glycoprotein 2. This conformational change plays an important role in the fusion of virus with endosomal membrane, a critical step in the EBOV entry (Das et al., 2020).

Likewise, Gunaratne et al. reported a critical role of TPCs in Middle East Respiratory Syndrome coronavirus (MERS-CoV) infections (Gunaratne et al., 2018). The knockdown of TPCs but not that of lysosomal transient receptor potential mucolipin-1 (TRPML1) led to impairment of MERS-CoV translocation via endo-lysosomes (Gunaratne et al., 2018). Importantly, the inhibition of NAADP-evoked lysosomal  $\text{Ca}^{2+}$  release via bisbenzyl-isoquinoline alkaloids blocked MERS pseudovirions translocation (Gunaratne et al., 2018). This highlights the therapeutic significance of targeting TPCs in regulating MERS-CoV pathogenesis. Recently, TPC1/2 were reported to play a critical role in



**Fig. 5. Lysosomal  $\text{Ca}^{2+}$  dynamics and viral entry.** A variety of viruses target lysosomal  $\text{Ca}^{2+}$  efflux channels for host cell entry via endocytic route. Viruses such as EBOV, Marburg virus, Polyoma virus and MERS-CoV target TPCs for facilitating receptor mediated entry into host cells. Importantly, TPCs also regulate SARS-CoV-2 entry into host cells. While TRPML1 regulates HIV-1 replication and latent infection.

polyomavirus entry into the host cells (Dobson et al., 2020). Further, it was demonstrated that inhibition of TPC activity by tetrandrine abrogates Merkel cell polyomavirus and Simian virus 40 infection. In a timely and highly relevant study, Ou et al. demonstrated that human angiotensin converting enzyme 2 (hACE2) is the receptor for SARS-CoV-2, the virus responsible for current COVID-19 pandemic (Ou et al., 2020). The authors reported that SARS-CoV-2 enters cells primarily through endocytosis. Further, they showed that TPC2 but not TRPML1 plays a critical role in the SARS-CoV-2 entry. Importantly, Ou and colleagues demonstrated that inhibition of TPC2 by tetrandrine leads to decrease in SARS-CoV-2 pseudovirions entry (Ou et al., 2020). Therefore, further investigations focused on evaluating the efficacy of TPC targeting drugs as novel antiviral therapy are need of the hour.

Apart from TPCs, an important role for another endo-lysosomes localized  $\text{Ca}^{2+}$  channel TRPML1 is reported in HIV-1 infection (Khan et al., 2019). As discussed in previous sections, HIV-1 Tat protein is critical for HIV-1 replication and latent infection. The degradation of Tat protein depends on pH of host cell endo-lysosomal compartment. Khan et al. demonstrated that acidification of endo-lysosomes by inducing TRPML1 activation leads to Tat degradation (Khan et al., 2019). This suggests that TRPML1 could be a potential therapeutic candidate against latent HIV-1 infections (Khan et al., 2019).

Taken together, these studies highlight that lysosomal  $\text{Ca}^{2+}$  signaling is critical for viral entry. It is important to note that TPC2 but not TRPML1 is a promising target for EBOV, SARS-CoV-2 and MERS-CoV infections whereas TRPML1 plays a critical regulatory role in case of HIV-1 infections thereby suggesting viral specific targeting of lysosomal  $\text{Ca}^{2+}$  handling machinery (Please refer Fig. 5 for the diagrammatic summary). Therefore, these studies demonstrate that different viruses target specific endo-lysosomal  $\text{Ca}^{2+}$  channels for their entry into host cells. Notably, FDA-approved drugs targeting lysosomal  $\text{Ca}^{2+}$  dynamics can robustly decrease viral infections thereby suggesting that these drugs should be further evaluated in clinical trials for better management of viral diseases including current COVID-19 pandemic.

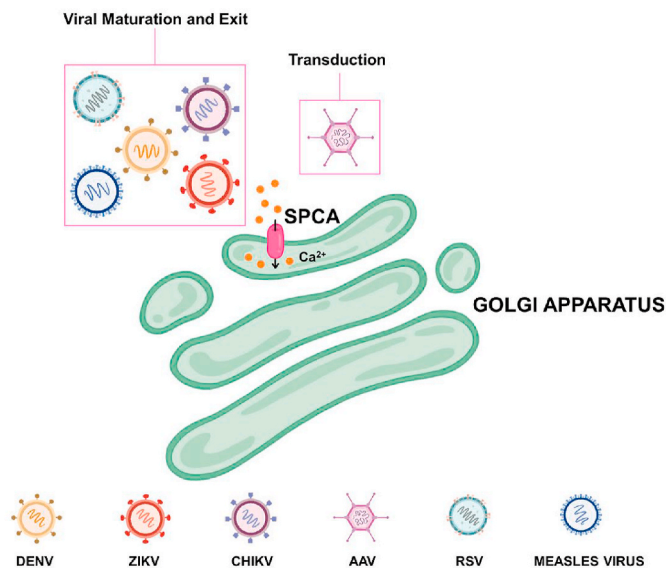
## 8. Disruption of the Golgi $\text{Ca}^{2+}$ signaling and viral infections

GA plays a critical role in post-translational modification and sorting of lipids and proteins. Further, GA is actively involved in intracellular

$\text{Ca}^{2+}$  dynamics. It is equipped with  $\text{Ca}^{2+}$  release channels,  $\text{Ca}^{2+}$  pumps and  $\text{Ca}^{2+}$  binding proteins. This  $\text{Ca}^{2+}$  handling toolkit is involved in regulating the spatio-temporal complexity of the cellular  $\text{Ca}^{2+}$  signaling (Pizzo et al., 2011). The GA  $\text{Ca}^{2+}$  handling machinery includes  $\text{IP}_3\text{R}$ , SERCA, RyR and SPCA1. SPCA1 is a critical regulator of GA  $\text{Ca}^{2+}$  content and plays an important role in the maintenance of GA ultrastructure and in the sorting of proteins to the PM.

The respiratory syncytial virus (RSV) is a RNA virus belonging to Paramyxoviridae family (Collins et al., 2013). Recently, Hoffmann et al. performed a genome-wide knockout screen in human haploid cells to identify host factors necessary for RSV infection (Hoffmann et al., 2017). Interestingly, SPCA1 was one of the most promising hits in this screening as it regulates processing of RSV glycoproteins and maturation of the virus (Hoffmann et al., 2017). Importantly, SPCA1 KO robustly decreased RSV infection and spread as viral glycoproteins critical for viral infectivity are not processed in absence of SPCA1. In addition to RSV, SPCA1 is required for infection of numerous other RNA viruses such as measles, dengue, zika, and chikungunya viruses (Hoffmann et al., 2017). Taken together, SPCA1 is emerging as a promising target for stalling spread of RNA viruses.

Parvoviruses such as adeno-associated viruses (AAVs) possess a single-stranded DNA genome (Madigan et al., 2020). AAV2 hijacks endosomal channels to migrate from the cell surface to the nucleus, including several endosome-trafficking proteins such as VPS29, VPS52, VPS53, VPS54, SPCA1 (Pillay et al., 2016). These proteins are engaged in the retrograde transport from endosomes to GA (Pillay et al., 2016). SPCA1 mediated GA  $\text{Ca}^{2+}$  homeostasis plays a critical role in AAV transduction (Madigan et al., 2020). The authors generated a SPCA1 CRISPR knockout (KO) line and demonstrated that AAV transduction is reduced upon SPCA1 KO (Madigan et al., 2020). Interestingly, SPCA1 KO does not inhibit AAV binding, its cellular uptake or nuclear entry, but SPCA1 KO cells exhibit scattered and punctuated trafficking (Madigan et al., 2020). Additionally, SPCA1 KO leads to significant inhibition of transcription of the vector genome. This SPCA1 KO phenotype and AAV transduction was rescued by the  $\text{Ca}^{2+}$  chelator BAPTA-AM, which decreases cytosolic  $\text{Ca}^{2+}$ . Conversely, AAV transduction is inhibited by the  $\text{Ca}^{2+}$  ionophore ionomycin, which enhances cytosolic  $\text{Ca}^{2+}$  levels (Madigan et al., 2020). Collectively, these data suggest that SPCA1 mediated modulation of cytosolic  $\text{Ca}^{2+}$  concentration is a key regulator



**Fig. 6.** Golgi  $\text{Ca}^{2+}$  signaling and viral exit. Viruses frequently target Golgi  $\text{Ca}^{2+}$  signaling for facilitating their maturation and exit from the host cells. For example, DENV, ZIKV, CHIKV, RSV and Measles virus act on Golgi localized SPCA for enabling maturation of their glycoproteins and for their exit from the host cells. Further, SPCA regulated Golgi and cytosolic  $\text{Ca}^{2+}$  homeostasis plays a critical role in AAV transduction.

of AAV transduction. Collectively, the above-discussed studies shed light on the critical role of GA  $\text{Ca}^{2+}$  homeostasis and establish SPCA1 as an important regulator of viral infections (Also refer pictorial summary in Fig. 6).

### 9. Targeting host cell $\text{Ca}^{2+}$ signaling for curtailing viral pathogenesis

As discussed in above respective sections, viruses dysregulate intracellular  $\text{Ca}^{2+}$  homeostasis by targeting different cellular  $\text{Ca}^{2+}$  compartments (ER, PM, mitochondria, Golgi and lysosomes) for promoting viral pathogenesis. Since viruses interfere in the functioning of the  $\text{Ca}^{2+}$  handling machinery for its benefit, it is not surprising that host cell  $\text{Ca}^{2+}$  signaling toolkit has emerged as a promising target for clinical management of viral pathogenesis.

In a highly relevant study, Wang et al. screened FDA-approved drugs to target JEV (Wang et al., 2017). A high-throughput screening of FDA-approved drugs for inhibitors of JEV led to identification of 5 drugs, including 3 VGCCs blockers (manidipine, cilnidipine, and benidipine hydrochloride). Further, the authors demonstrated that Verapamil (another VGCCs inhibitor) treatment leads to dose dependent inhibition of JEV in human hepatocellular carcinoma (Huh-7) and African Green monkey kidney (Vero) cells. Moreover, VGCCs blockers inhibited replication of a variety of other flaviviruses such as ZIKV, DENV and WNV. Significantly, manidipine protects mice against JEV-induced lethality by reducing the viral load in the brain. This study clearly demonstrates clinical relevance VGCC inhibitors in targeting flavivirus infection (Wang et al., 2017). A similar study had earlier identified the FDA-approved VGCC blockers amiodarone and verapamil as potent inhibitors of filovirus entry in the host cells (Gehring et al., 2014). The experiments conducted in this study clearly demonstrated that these drugs act on host cells rather than the viruses directly (Gehring et al., 2014). Similarly, benidipine and nifedipine were demonstrated to curtail severe fever with thrombocytopenia syndrome virus (SFTSV) entry and replication (Li et al., 2019). Notably, both benidipine and nifedipine reduced SFTSV induced mortality in humanized mice model. Interestingly, authors performed a retrospective clinical study on the SFTSV patients data and suggested that nifedipine treatment

significantly decreases the mortalities associated with SFTSV infection (Li et al., 2019). More recently, verapamil was reported to inhibit infection of polyomaviruses such as Simian virus 40 and Merkel cell polyomavirus (Dobson et al., 2020). Taken together, these studies highlight the potential benefit of targeting host cell VGCC for stalling viral infections.

Recently, Nathan et al. reported that  $\text{Ca}^{2+}$  interacts with EBOV fusion peptide (FP) and promotes the viral infection (Nathan et al., 2020). The authors demonstrated that  $\text{Ca}^{2+}$  promotes EBOV infection via direct interaction of  $\text{Ca}^{2+}$  with FP of EBOV. The glycoprotein residues D522 and E540 in the FP of EBOV are critical for  $\text{Ca}^{2+}$  interaction. By performing biophysical assays and spectroscopic studies, it was determined that interaction of  $\text{Ca}^{2+}$  with FP results in alteration in lipid ordering within host membrane. Further, by utilizing circular dichroism spectroscopy it was revealed that  $\text{Ca}^{2+}$  interaction with FP leads to conformational change (via promoting  $\alpha$ -helical structure of the fusion peptide). This conformational change increases the membrane fusion and viral infectivity. Therefore, implicating the potential of FDA-approved  $\text{Ca}^{2+}$  interfering drugs in combating the viral infections (Jayaseelan and Paramasivam, 2020; Nathan et al., 2020). Indeed, a screening of around 2600 FDA-approved drugs and molecular probes identified a VGCC blocker Bepridil as one of the most potent inhibitor of EBOV entry in human cells *in vitro* (Johansen et al., 2015). Furthermore, *in vivo* experiments in mouse infection model of EBOV corroborated the efficacy of Bepridil in curtailing EBOV infection and associated pathogenesis. In a highly timely study relevant to the current COVID-19 pandemic, Vatansever et al. demonstrated that Bepridil inhibits SARS-CoV-2 infection in human cells (Vatansever et al., 2021). It was shown that Bepridil blocks both entry and infection of SARS-CoV-2 by modulating endo-lysosomal pH and by inhibiting key protease of SARS-CoV-2 respectively. Taken together, these studies elegantly demonstrate the potential clinical significance of FDA-approved VGCC inhibitor Bepridil in stalling SARS-CoV-2 and EBOV infections. Certainly, in current COVID-19 pandemic situation Bepridil must be further evaluated for its efficacy in controlling SARS-CoV-2 infection.

In an exciting study, Hyser lab recently demonstrated that rotaviral infection leads to a paracrine signaling resulting in generation of inter-cellular  $\text{Ca}^{2+}$  waves (ICWs) (Chang-Graham et al., 2020). Chang-Graham et al. showed that rotaviral infected cells secrete adenosine 5'-diphosphate (ADP) that activates P2Y1 purinergic receptors on neighboring uninfected cells, which in turn leads to rise in cellular  $\text{Ca}^{2+}$  concentration. The authors showed that P2Y1 knockout abrogates ICWs generation and viral replication. Importantly, using neonatal mice model, it was reported that P2Y1 inhibition significantly reduces severity of RV induced diarrhea *in vivo* (Chang-Graham et al., 2020). In the future, it would be pertinent to identify FDA-approved drug/s that can inhibit P2Y1 and evaluate their safety and efficacy in treating clinical rotaviral infections.

As discussed earlier, a large number of viruses attack mitochondrial  $\text{Ca}^{2+}$  signaling for either meeting higher energetic requirements or for inducing host cell apoptosis. Interestingly, Perocchi lab performed an orthogonal interspecies chemical screening in yeast mitochondria and human cells for identifying modulators of MCU (Arduino et al., 2017). Arduino et al. screened more than 600 small molecules belonging to NIH clinical collection library to eventually identify mitoxantrone (an FDA-approved drug) as a highly specific MCU inhibitor (Arduino et al., 2017). Similarly, a high throughput screening of around 120,000 compounds identified DS16570511 as a novel inhibitor of MCU activity in a variety of cells and beating rat heart (Kon et al., 2017). Subsequently, work from Madesh lab reported synthesis and characterization of Ru265 (Woods et al., 2019). The authors reported that Ru265 is highly specific inhibitor of MCU activity. Importantly, Woods et al. demonstrated that Ru265 is cell permeable and nominally toxic molecule that does not perturb cytosolic  $\text{Ca}^{2+}$  homeostasis and mitochondrial membrane potential. More recently, a high throughput screening of over 44,000 non-proprietary small molecules was performed for characterizing the

**Table 1**  
The outcomes of viral interaction with host cell Ca<sup>2+</sup> handling machinery.

Site	S. No.	Virus	Viral Protein	Host target	Effect on host cell Ca <sup>2+</sup> signaling	Relevance to viral pathogenesis	Reference
PM	1	IAV	Hemagglutinin (HA)	VGCC	Induces intracellular Ca <sup>2+</sup> oscillation	Helps in viral entry and replication	<a href="#">Fujioka et al. (2018)</a>
	2	HIV-1	Tat	VGCC	Elevation of intracellular Ca <sup>2+</sup>	Host immune dysfunction	<a href="#">Zocchi et al. (1998)</a>
	3	Flavivirus (JEV, ZIKV, DENV, YFV and WNV)	NS4B	VGCC	Increases cytosolic Ca <sup>2+</sup>	Viral replication	<a href="#">Wang et al. (2017)</a>
	4	NWA	Viral glycoproteins (GP)	VGCC	Increases cytosolic Ca <sup>2+</sup>	Helps in viral entry and infection	<a href="#">(Lavanya et al., 2013; Sarute and Ross, 2020)</a>
	5	HBV	HBx	Orai1	Increases cytosolic Ca <sup>2+</sup>	Essential for HBV replication	<a href="#">(Yao et al., 2018)</a>
	6	HTLV-1	p12I	SOCC	Elevation of cytoplasmic Ca <sup>2+</sup>	Aids in viral replication	<a href="#">Ding et al. (2002)</a>
	7	RV	NSP4	SOCC	SOCE activation, increases cytoplasmic Ca <sup>2+</sup> levels	Aid in viral replication	<a href="#">(Díaz et al., 2012; Hyser et al., 2013)</a>
	8	DENV	Not known	Orai1	Rise in cytosolic Ca <sup>2+</sup>	Viral replication	<a href="#">Dionicio et al. (2018)</a>
	9	Arenavirus and Filovirus	VP40	Orai1	Rise in cytosolic Ca <sup>2+</sup> levels	Viral budding and spread	<a href="#">Han et al. (2015)</a>
	10	HIV-1	Gp120, Tat	NMDAR	Increases intracellular Ca <sup>2+</sup>	Not known	<a href="#">(Nath et al., 1995; Self et al., 2004)</a>
	11	HCV, DEVN, ZIKV	Not Known	TRPV4	Rise in cytosolic Ca <sup>2+</sup>	Viral replication and exit	<a href="#">Doñate-Macián et al. (2018)</a>
	12	HBV	HBx	PMCA	Elevation of the cytosolic Ca <sup>2+</sup>	Enhances HBV DNA replication and HBV core assembly	<a href="#">Zhou et al. (2013)</a>
	ER	13	RV	Not Known	NCX	Rise in cytosolic Ca <sup>2+</sup>	RV replication
1		RV	NSP4	Acts as ER Viroporin	Depletes ER Ca <sup>2+</sup> and increase in cytosolic Ca <sup>2+</sup>	Viral replication and assembly	<a href="#">(Díaz et al., 2008; Hyser et al., 2013; Pham et al., 2017)</a>
2		HCMV	US21	Acts as ER Viroporin	Depletes ER Ca <sup>2+</sup> stores	Decreases cell susceptibility to apoptosis	<a href="#">Luganini et al. (2018)</a>
3		HCMV	UL37	IP <sub>3</sub> R	Mobilizes Ca <sup>2+</sup> from ER to cytosol	Enhanced ATP production to meet viral energy demands	<a href="#">Sharon-Friling et al. (2006)</a>
4		HCMV	US28	Triggers PLC-β signaling	Release of intracellular Ca <sup>2+</sup>	May have role in latent infection	<a href="#">Miller et al. (2012)</a>
5		HCMV	vCXCL1	Agonist for human chemokines CXCR1 and CXCR2	Intracellular Ca <sup>2+</sup> release	Viral dissemination through the host	<a href="#">(Penfold et al., 1999; Wang et al., 2003)</a>
6		EBV	LMP-1	SERCA3	Increase in resting cytosolic Ca <sup>2+</sup> levels	Development of latent, persistent infection and cell immortalization	<a href="#">Dellis et al. (2009)</a>
7		HIV	Gag	IP <sub>3</sub> R	Ca <sup>2+</sup> release from ER	Viral budding, formation of VLPs	<a href="#">(Ehrlich et al., 2011, 2014; Ehrlich and Carter, 2012)</a>
8		HIV	Nef	IP <sub>3</sub> R	Ca <sup>2+</sup> release from ER	AIDS pathogenesis	<a href="#">(Ehrlich et al., 2010; Manninen and Saksela, 2002)</a>
9		HBV	HBx	IP <sub>3</sub> R	Increase in mitochondrial Ca <sup>2+</sup> uptake	Aid in viral replication	<a href="#">Yang and Bouchard (2012)</a>
Mitochondria	10	HSV	Not defined	IP <sub>3</sub> R	Increases cytosolic Ca <sup>2+</sup>	Viral penetration, delivery of viral capsids to host cell's nucleus.	<a href="#">(Cheshenko et al., 2003, 2007)</a>
	1	HBV	HBx	mPTP	Releases Ca <sup>2+</sup> from mitochondria	Regulates HBV replication	<a href="#">Bouchard et al. (2001)</a>
	2	HCV	NS5A	MCU	Increased mitochondrial Ca <sup>2+</sup> influx and nuclear translocation of transcription factors	Helps in viral pathogenesis	<a href="#">Gong et al. (2001)</a>
	3	HCV	HCV polyprotein	MCU	Increase in mitochondrial Ca <sup>2+</sup> influx	May induce apoptosis	<a href="#">Piccoli et al. (2007)</a>
	4	HCV	Core protein	MCU	Increase in mitochondrial Ca <sup>2+</sup> uptake	Increases ROS production and alters apoptosis	<a href="#">Li et al. (2007)</a>
	5	HEV	Orf3	VDAC1	Upregulation of VDAC	Enhances viral replication	<a href="#">Moin et al. (2007)</a>
	6	HIV-1	Tat	MCU	Increase in mitochondrial Ca <sup>2+</sup> uptake	Host cell apoptosis	<a href="#">(Kruman II et al., 1998)</a>
	7	HIV-1	Tat	VDAC	Rise in mitochondrial Ca <sup>2+</sup>	Host cell apoptosis	<a href="#">Lecoeur et al. (2012)</a>
Lysosomes	8	PV	Not known	VDAC and MCU	Increase mitochondrial Ca <sup>2+</sup> levels	Apoptosis induction	<a href="#">Brisac et al. (2010)</a>
	1	EBOV and Marburgvirus	Not known	TPC2	Ca <sup>2+</sup> release from lysosomes	EBOV entry and infection, Marburgvirus infection	<a href="#">(Penny et al., 2019; Sakurai et al., 2015; Simmons et al., 2016)</a>

(continued on next page)

Table 1 (continued)

Site	S. No.	Virus	Viral Protein	Host target	Effect on host cell Ca <sup>2+</sup> signaling	Relevance to viral pathogenesis	Reference
	2	Polyomavirus	Not known	TPC1/2	Ca <sup>2+</sup> release from lysosomes	Viral entry	Dobson et al. (2020)
	3	MERS-CoV	Furin	TPCs	Ca <sup>2+</sup> release from lysosomes	Viral entry	Gunaratne et al. (2018)
	4	SARS-CoV-2	Not known	TPC2	Ca <sup>2+</sup> release from lysosomes	Viral entry	Ou et al. (2020)
	5	HIV-1	Tat	TRPML1	Ca <sup>2+</sup> release from lysosomes	HIV-1 replication and latent infections	Khan et al. (2019)
Golgi	1	DENV, ZIKV, CHIKV, RSV and Measles virus	Not known	SPCA1	–	Viral maturation and exit	Hoffmann et al. (2017)
	2	AAV	Not known	SPCA1	Perturbs cytosolic Ca <sup>2+</sup>	AAV transduction	(Madigan et al., 2020; Pillay et al., 2016)

inhibitors of mitochondrial Ca<sup>2+</sup> uptake 1 (MICU1), which regulates mitochondrial Ca<sup>2+</sup> influx (Di Marco et al., 2020). This arduous study identified 2 lead molecules that authors named as MCU-i4 and MCU-i11. Both of these molecules were able to efficiently and specifically inhibit MICU1 thereby decreasing mitochondrial Ca<sup>2+</sup> concentration in *in vitro* and *ex vivo* assays (Di Marco et al., 2020; Nathan et al., 2020). Taken together, these recent studies have led to identification and characterization of highly specific small molecules that can target mitochondrial Ca<sup>2+</sup> handling machinery. Considering the critical role of mitochondrial Ca<sup>2+</sup> dynamics in viral infections, it would be interesting to evaluate the efficacy of these lead compounds in managing viral pathogenesis.

The emerging literature suggests that lysosomal Ca<sup>2+</sup> dynamics, especially lysosomal Ca<sup>2+</sup> efflux channel TPC2 plays a critical role in viral entry (please refer lysosomal Ca<sup>2+</sup> signaling section for details). Excitingly, TPC2 inhibition is shown to stall entry of SARS-CoV-2, virus responsible for COVID-19 pandemic, as well (Ou et al., 2020). Recently, 1500 FDA-approved drugs were screened for inhibiting TPC2 activity (Penny et al., 2019). The authors identified and characterized two drugs that can inhibit endogenous NAADP-evoked lysosomal Ca<sup>2+</sup> release and TPC2 channel activity. Significantly, these drugs lead to substantial reduction in EBOV infection in HeLa cells (Penny et al., 2019). In the future, it would be worth to investigate the potential of repurposing these FDA-approved drugs for clinical management of viral infections. Certainly, clinical trials for assessing the safety and efficacy of these drugs in restricting viral pathogenesis would be required to reveal the real potential of these drugs.

## 10. Future perspectives

In this review, we have discussed the dysregulation of host cell Ca<sup>2+</sup> homeostasis during viral infections. Several viral proteins target specific cellular Ca<sup>2+</sup> compartments (ER, PM, mitochondria, GA and lysosomes) for promoting viral pathogenesis. Viral proteins such as Tat and gp120 of HIV-1, HBx of HBV etc. modulate functioning of PM localized Ca<sup>2+</sup> channels and pumps. Similarly, viral proteins like Gag and Nef of HIV, HBx of HBV etc. induce Ca<sup>2+</sup> release from ER to cytosol (Please refer Table 1 for the detailed list of viral proteins, their host cell targets and the resulting effect on cellular Ca<sup>2+</sup> homeostasis). Although these proteins have been reported to modulate functioning of host cell Ca<sup>2+</sup> handling proteins, the detailed molecular choreography that connects viral infections to perturbations in Ca<sup>2+</sup> homeostasis remains poorly understood. Likewise, how changes in cytosolic and/or organellar Ca<sup>2+</sup> aid in viral life cycle progression and pathogenesis remains poorly understood. With the advent of highly specific genetically encoded Ca<sup>2+</sup> sensing probes coupled with super resolution microscopy and systems biology approaches, the stage is set for dissecting out the intricate signaling cascades working at the intersection of viral infections and host cell Ca<sup>2+</sup> dynamics.

As discussed in the lysosomal Ca<sup>2+</sup> dynamics and viral entry section, a variety of viruses target lysosomal Ca<sup>2+</sup> channels especially TPCs for entry into host cells. Going forward, it would be worth to examine the

role of TPCs in non-viral infectious diseases caused by the pathogens that target endo-lysosomes for driving infections. Since FDA-approved drugs targeting TPCs have been recently identified, their further evaluation and efficacy in clinical settings is required.

The current COVID-19 pandemic has severely affected whole world with over 3 million deaths. Studies have reported that Ca<sup>2+</sup> is required for the fusion of MERS-CoV, SARS-CoV and Rubella virus with the host cells suggesting that Ca<sup>2+</sup> plays a critical role in these viral infections (Dubé et al., 2014; Lai et al., 2017; Straus et al., 2020b). Interestingly, based on structural homology, bioinformatics and meta-analyses, it is suggested that Ca<sup>2+</sup> could act as an important regulator of SARS-CoV-2 entry into host cells (Cashman, 2020a, 2020b). Further, TPCs have been recognized as targetable option for stalling SARS-CoV-2 entry into host cells (Filippini et al., 2020; Ou et al., 2020; Zhao et al., 2021) and TPC antagonist tetrandrine is hypothesized as a potential antiviral against SARS-CoV-2 (Heister and Poston, 2020). Indeed, there is an ongoing clinical trial that is evaluating efficacy of tetrandrine in COVID-19 treatment (<https://clinicaltrials.gov/ct2/show/study/NCT04308317>). Moreover, Bepridil a VGCC inhibitor is shown to inhibit SARS-CoV-2 infection *in vitro* (Vatansever et al., 2021). Likewise, FDA-approved VGCC blockers amlodipine and nifedipine have been shown to stall SARS-CoV-2 entry into host cells (Straus et al., 2020a). Taken together, these studies have highlighted a critical role of Ca<sup>2+</sup> dynamics in COVID-19 infection. In light of current pandemic, further investigations on VGCC blockers and TPC inhibitors as potential antivirals against SARS-CoV-2 are urgently needed.

Further, as discussed earlier VGCC inhibitors have shown promising role in curtailing flavivirus and polyomavirus infections. It is important to note that VGCC inhibitors are routinely prescribed for managing hypertension and they are part of long term medication for hypertensive patients. Therefore, it would be relevant to conduct epidemiological surveys of the patients taking VGCC inhibitors for possible protection against flavivirus, SARS-CoV-2 and polyomavirus infections. Such surveys will most likely provide insightful information that may assist in designing future antiviral therapies.

In summary, several elegant studies have demonstrated that Ca<sup>2+</sup> handling proteins are attractive therapeutic targets for stalling a variety of viral infections. With availability of both FDA-approved drugs and bio-active lead molecules targeting these proteins, it is an area that demands immediate and critical attention for managing viral infections. Certainly, preclinical safety and efficacy studies in relevant animal models would be needed before testing these drugs in clinical trials. In the current scenario wherein outbreaks of viral infections are frequently observed, it is actually need of the hour to investigate the clinical significance of targeting host cell Ca<sup>2+</sup> signaling for curtailing viral pathogenesis.

## Declaration of competing interest

Authors report no conflicts of interest.

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