Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Cell Calcium 94 (2021) 102360

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Review

Lysosomal ion channels involved in cellular entry and uncoating of enveloped viruses: Implications for therapeutic strategies against SARS-CoV-2

Zhuangzhuang Zhao, Pan Qin, Yao-Wei Huang *

Key Laboratory of Animal Virology of Ministry of Agriculture, Institute of Preventive Veterinary Medicine, Department of Veterinary Medicine, Zhejiang University, Hangzhou 310058, China

ARTICLE INFO

Keywords:
TPC2
TRPML2
PI(3,5)P2
Lysosome
Enveloped viruses
SARS-CoV-2

ABSTRACT

Ion channels are necessary for correct lysosomal function including degradation of cargoes originating from endocytosis. Almost all enveloped viruses, including coronaviruses (CoVs), enter host cells via endocytosis, and do not escape endosomal compartments into the cytoplasm (via fusion with the endolysosomal membrane) unless the virus-encoded envelope proteins are cleaved by lysosomal proteases. With the ongoing outbreak of severe acute respiratory syndrome (SARS)-CoV-2, endolysosomal two-pore channels represent an exciting and emerging target for antiviral therapies. This review focuses on the latest knowledge of the effects of lysosomal ion channels on the cellular entry and uncoating of enveloped viruses, which may aid in development of novel therapies against emerging infectious diseases such as SARS-CoV-2.

1. Introduction

Viruses are made up of highly condensed nucleic acids (RNA or DNA) surrounded by a protective protein coat. The presence or absence of a host-derived lipid membrane envelope features strongly in the taxonomic classification of viruses, and many important enveloped viruses have been widely studied including the families *Coronaviridae*, *Filoviridae* (Ebola virus; EBOV) and *Orthomyxoviridae* (influenza virus). The envelope of such viruses contains virus-encoded proteins that are essential for binding and entry into host cells. The coronavirus (CoV) spike (S) glycoprotein, consisting of S1 and S2 subunits, exists on the virion surface as a trimer [1,2]. The envelope of EBOV contains a metastable trimer of glycoprotein (GP) obtained during budding from cells, which is the primary determinant of viral entry [3]. Some viruses even have both "naked" and enveloped forms, such as hepatitis E virus (HEV; family *Hepeviridae*) and hepatitis A virus (HAV; family *Picornaviridae*) [4,5]; these "quasi-enveloped" viruses enter cells in a way similar to that of enveloped viruses. For example, HAV particles cloaked in host membranes can enter cells through endocytosis, where enzymatic degradation in the late endosomes leads to uncoating [6]. In certain conditions, infection of the jejunum and ileum may be facilitated by digestive enzymes in the intestinal lumen, wherein enveloped viruses can fuse with host cell membranes directly, releasing the genetic material into the cytoplasm [7,8]. However, under normal conditions, almost all enveloped viruses enter host cells via endocytosis. Further study of the endolysosomal cues that trigger cellular entry and uncoating of enveloped viruses is essential for development of broad-spectrum antiviral strategies against such emerging pathogens as SARS-CoV-2.

2. Cellular entry and uncoating of enveloped viruses

In order for an enveloped virus to establish a productive infection, it must overcome cellular barriers to deliver its genetic materials to the cytoplasm. The process of viral entry includes viral attachment to cells, intracellular trafficking, and delivery of the viral genome. Almost all enveloped viruses enter host cells via specific interactions with receptor proteins which trigger endocytosis pathways such as macro- or micropinocytosis, or by induction of clathrin coat formation [9–12]. Once enveloped viruses are taken up, they are transported by the vesicular system via membrane trafficking and processed from early to late endosomes, with a gradual drop in pH, Rab-switching, transport to perinuclear regions, and eventually routed towards degradative organelles known as lysosomes (see Fig. 1). When a virus reaches an appropriate point of the pathway, viral envelope fusion with the endolysosomal membrane will create a fusion pore within the

* Corresponding author at: Zhejiang University, China.
E-mail address: yhuang@zju.edu.cn (Y.-W. Huang).

https://doi.org/10.1016/j.ceca.2021.102360
Received 4 August 2020; Received in revised form 13 January 2021; Accepted 15 January 2021
Available online 23 January 2021
0143-4160/© 2021 Elsevier Ltd. All rights reserved.

endosomal membrane, allowing its genome to be released into the cytosol for downstream viral replication processes. This entire process of viral uncoating also requires assistance from cellular factors [13].

Uncoating is an obligatory second step for virus infection, which makes it an attractive antiviral target. Viral envelope fusion with the endolysosomal membrane depends on cellular factors, such as intracellular receptors. In the same way that viruses need cell surface receptors to bind to cells, inside the endosome, viruses also require an intracellular receptor to bind and trigger membrane fusion. All known filoviruses employ NPC1 (NPC intracellular cholesterol transporter 1) [14–16], and Lassa virus uses lamp1 (lysosomal associated membrane protein 1) [17] as an internal receptor to trigger uncoating (see Fig. 1). Other cellular factors such as the lysosomal TPC2 (two-pore channel 2) induce the fusion process and release of viruses from the endolysosomal compartments to the cytoplasm [18]. TPC2 has also been shown to be required for release of the SARS-CoV-2 genome into target cells [19], although the specific role of TPC2 in virus escape into the cytoplasm is not completely clear [20–23]. Another lysosomal ion channel, TRPML2 (the second member of the mammalian mucolipin TRP channel subfamily), can affect viral entry by enhancing the efficiency of viral trafficking in the endosomal system [24]. The following sections will go into greater detail on the endolysosomal environment needed for cellular entry and uncoating of enveloped viruses (see the summary in Table 1).

3. Lysosomal ion channels involved in cellular entry of virus

3.1. The lysosomal ion environment is crucial for lysosome function

Lysosomes are the cell's degradation and recycling center, where macromolecules are degraded by hydrolytic enzymes activated by low pH [25]. Lysosomes are known to be a dynamic hub for cellular metabolism, nutrient sensing, plasma membrane repair, secretion, and spatiotemporal intracellular signaling [26–28]. These vital cellular functions hinge on the precise control of ionic (Na^+, K^+, Ca^{2+}, Cl^-, H^+) gradients [29]. One can speculate that the coming years will bring many more examples of direct relationships between ion flux and endocytic function [30]. Lysosomes are the end-point of endocytosis, fed by the process of endosome maturation, and an understanding of the lysosomal ion environment will help to shed light on the entry process of enveloped

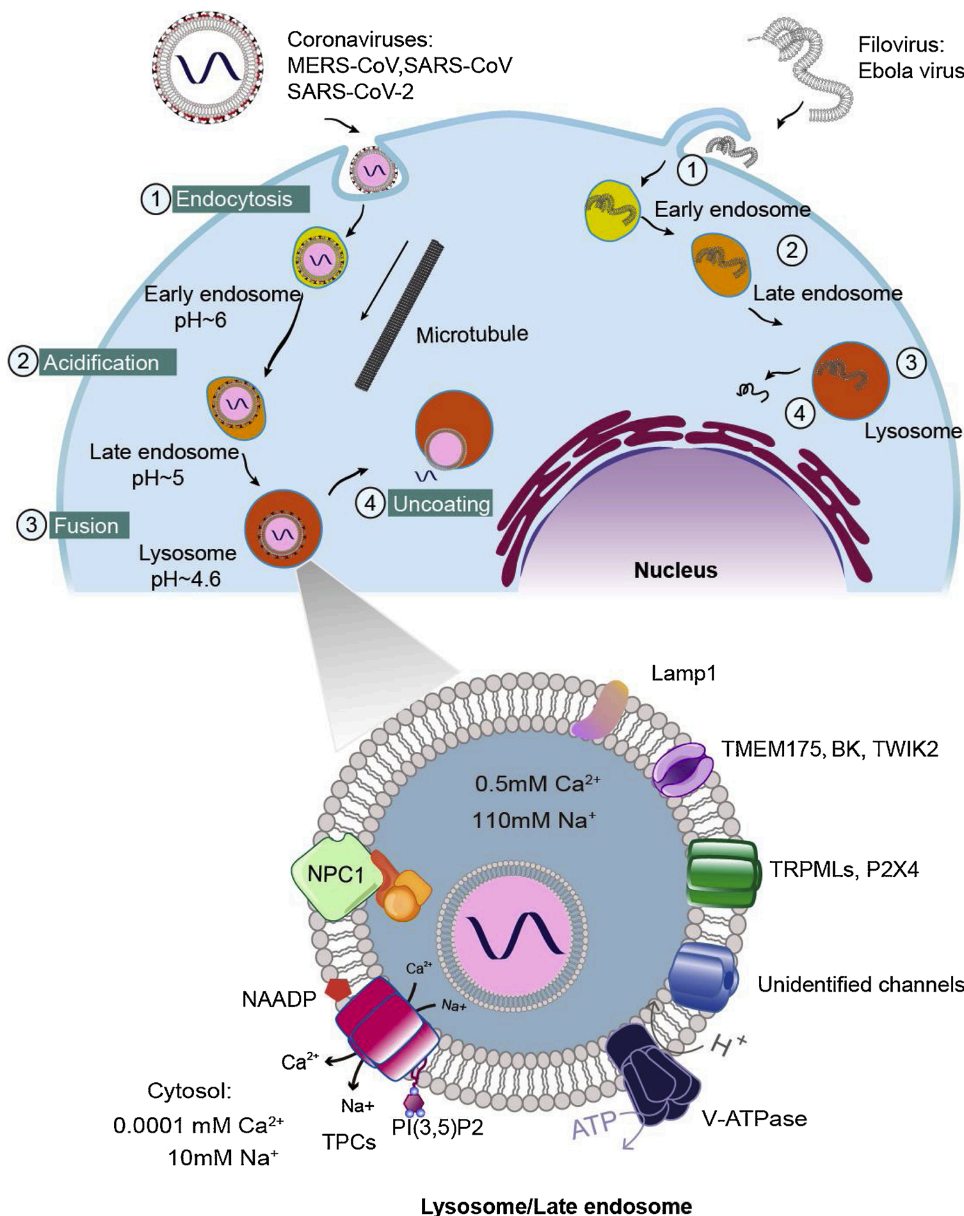

Fig. 1. Schematic representation of cellular entry and uncoating of enveloped viruses. (1) Cells present multiple endocytic pathways, allowing for endocytosis of small (coronaviruses, 80-120 nm in diameter, clathrin/caveolae-dependent/independent) and large (filovirus, 650 and 1400 nm in length, macropinocytosis dependent) viruses. (2) Despite the fact that enveloped viruses have various endocytic pathways, once inside the endosome system, the following steps are largely conserved as they are processed from early endosomes to late endosomes with a gradual drop in pH, and the viral spike glycoprotein is cleaved by low pH-activated proteases. (3) Cleaved spike glycoproteins are then able to interact with a specific intracellular receptor such as NPC1 (Ebola virus) or lamp1 (Lassa virus), which leads to spike glycoprotein-mediated fusion between the viral envelope and the endo-lysosomal membrane, and lysosome ion channels (TPCs and TRPML2) help trigger the fusion process. TPCs and TRPMLs are potential Ca^{2+} release channels in lysosomes. The H^+ gradient in the lysosome is established and maintained by V-ATPases. (4) The fusion process causes viral uncoating, releasing the viral genome into the cytoplasm for transcription and replication. Abbreviations: MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; LAMP1, lysosomal associated membrane protein 1; TMEM175, transmembrane protein 175; BK, the large Ca^{2+}-activated K^+ channel; K2P, two-pore domain potassium channels; TRPMLs, the mucolipin subfamily of transient receptor potential (TRP) cation channels; P2 × 4, purinergic receptor P2X, ligand-gated ion channel 4; PI(3,5)P2, phosphatidylinositol-3,5-bisphosphate; TPCs, two-pore channels; NAADP, nicotinic acid adenine dinucleotide phosphate; NPC1, Niemann-Pick type C1 protein.

Table 1
Lysosomal ion channels and enveloped viruses.

Lysosome ion channels	Ion flux	Subcellular localization	Antiviral activity	Lysosome function	Role in virus infection	References
TPC1	Na^+, Ca^{2+}	Early endosome, late endosome, lysosome	Ebola virus, Marburg virus, MERS-CoV	Endolysosomal excitability; pH homeostasis; ER– endosome membrane contact site formation	Promotes the fusion of virus and endosome;	[18,79]
TPC2	Na^+, Ca^{2+}	Late endosome, lysosome	SARS-CoV-2, Ebola virus, Marburg virus, MERS-CoV	pH homeostasis; Lysosome trafficking regulation	Promotes the fusion of virus and endosome;	[18,19,79]
TRPML1	Ca^{2+}	Late endosome, lysosome	No effect on SARS-CoV-2 and MERS-CoV	Lysosomal exocytosis; Retrograde transport; Plasma membrane repair;	None	[19,79]
TRPML2	Na^+, K^+, Cs^+	Late endosome, lysosome, plasma membrane (PM)	Influenza A virus, Yellow fever virus, and Zika virus	Lysosome trafficking regulation	Promotes viral vesicular trafficking	[24]
TRPML3	Ca^{2+}	Early endosome, late endosome, lysosome, PM	Cytomegalovirus (according to NextBio Research Disease Atlas)	Exosome release	Not clear	http://www.nextbio.com
BK	K^+	Late endosome, PM	Modulators targeting BK has no effect on Bunyavirus?	Lysosome membrane potential	None	[53,55,59]
TMEM175	K^+	Early endosome, late endosome, lysosome	Cannot be inhibited by compounds that inhibit Bunyavirus	pH homeostasis; Lysosome membrane potential	None	[58,59]
TWIK2	K^+	Lysosome	Bunyavirus	Background K^+ currents in the endolysosomes	Influence virus endosomal trafficking	[57,59,60]

viruses.

The ion environment within lysosomes is difficult to assess by classical techniques such as patch clamp analysis due to their internal localization and small size. A technique called lysosomal patch clamp was developed for direct recording from isolated endolysosomes which were pharmacologically enlarged using vacuolin-1 [31,32]. This technique paved the way to study endolysosomal channels in their native membranes. A variety of lysosomal ion channels have been identified, such as two-pore channels (TPCs), TRPMLs and transmembrane protein 175 (TMEM175), though many channels have yet to be characterized, such as the unidentified H^+ 'leak' channel which may affect lysosomal pH via nutrient-sensitive cellular cues [33].

3.2. Lysosomal H^+ and V-ATPase

Lysosomes are characterized by an acidic lumen pH, which can reach as low as 4.6 [34]. This acidic environment can be attributed to a proton pump V-ATPase complex, which pumps H^+ from the cytoplasm into the endolysosomal lumen in an energy-dependent manner. The V-ATPase complex has been identified as a critical host factor for viral entry [35,36]. Inhibitors of V-ATPase such as bafilomycin A or other luminal pH-related lysosomotropic agents ammonium chloride (NH_4Cl) and chloroquine (which accumulate in acidic organelles such as endosomes and lysosomes and neutralize their pH) [37] display a wide-spectrum of antiviral effects against influenza and CoVs [19,38–40].

The role of H^+ in viral infection is closely related to the activities of endolysosomal proteases, which are required for cleavage of the viral S glycoprotein [41]. In addition to protease activities, a low pH also induces conformational changes in the viral fusion loop [42,43], which inserts into the endosomal membrane, pulling EBOV and endolysosomal membranes together [44].

3.3. Lysosomal Cl^-

Lysosomes are highly enriched in chloride ions (Cl^-), and the luminal [Cl^-] can reach 60–80 mM [45], maintained by the voltage-gated chloride channel 7 (CLC7) [46,47]. As Cl^- is the antagonistic ion of H^+ and other positively charged ions, Cl- channels help regulate lysosomal pH levels [46], lysosomal enzyme activity [45], Ca^{2+} release from the lysosome, and cell volume. Tamoxifen and NPPB, two Cl- channel regulators, have been found to block viral entry by inhibiting viral binding and penetration and by disrupting Ca^{2+} homeostasis [48]. Chloride intracellular channel 1 (CLIC1) has been shown to be required for regulation of endolysosomal pH, and silencing of CLIC1 decreased infection by hepatitis C virus (HCV) [49]. This may be due to the fact that an acidic late endosome/lysosome pH is crucial for uncoating of HCV. Since the acidic endolysosomal pH is crucial for induction of viral membrane fusion to allow genome release, lysosomal Cl^- channels are a potential target for antiviral strategies against a broad-spectrum of endocytic pathogens including SARS-CoV-2.

3.4. Lysosomal K^+/ Na^+

The lysosome lumen is generally thought to be high in K^+ and low in Na^+ [50,51], suggesting a lack of a Na^+ or K^+ concentration gradient across the lysosomal membrane. However, a recent study has challenged this view by showing the isolated lysosome fractions were low in [K^+] and high in [Na^+] in the lysosomal lumen [52]. Recent whole-endolysosome patch-clamp studies showing the presence of multiple Na^+- and K^+- selective channels in the lysosome [52–55]. The lysosomal Na^+ and K^+ gradients control various lysosomal functions, including lysosomal acidification and catabolite export [53,56]. K^+ was identified as a biochemical cue to activate the viral entry process, and K^+ channel inhibition can alter the distribution of K^+ across the endosomal system and arrest virus trafficking in endosomes [57].

3.4.1. Lysosomal K^+ channels: TMEM175 and BK channels

Recent studies have identified two lysosomal K^+ channels: TMEM175 and large conductance Ca^{2+}-activated K^+ (BK) channel [53,55,58]. TMEM175 is responsible for K^+ conductance in endosomes and lysosomes [54], which plays important roles in setting the lysosomal membrane potential ($\Delta\Psi$, defined as $V_{cytosol}$-V_{lumen}; V_{lumen} is set to 0 mV) [52,54] and maintaining pH stability. BK channels regulate $\Delta\psi$ of endolysosomes in both excitable and non-excitable cells [53,55]. TMEM175 and BK likely affect viral entry. A recent study showed that modulators targeting BK channels had no effect on Bunyavirus [59], but the modulator (Tram 34) used in the study is actually a potent blocker of the intermediate-conductance Ca^{2+}-activated K^+ (IKCa) channel rather than the BK channel. Thus, the conclusion that BK channels are not involved in Bunyavirus infection is not supported, and whether the BK channel in lysosomes is a potential antiviral target for SARS-CoV-2 or other enveloped viruses still lacks experimental evidence. Quinine and tetraethylammonium, which inhibit Bunyavirus, could not inhibit TMEM175 [57], although whether TMEM175 affects SARS-CoV-2

infection is still lacking experimental evidence.

Other lysosomal potassium channels: weakly inwardly rectifying K^+ channel 2 (TWIK2) is a K_2P channel expressed in lysosomes. TWIK2 generates functional background K^+ currents in the endolysosomes, and its expression affects the number and mean size of the lysosomes [60]. Kcnk6 (encoding TWIK2) and other K_2P channels are involved in Bunyavirus intrusion [59]. K_2P channels in viral infection are thought to influence virus endosomal trafficking, as their inhibition can alter the distribution of K^+ across the endosomal system and arrest virus trafficking in endosomes [57]. In addition to influencing virus endosomal trafficking, K_2P channels also effect conformational changes in the viral fusion loop. As mentioned above, K_2P channels are involved in endosomal K^+ accumulation, and some studies have shown that exposure of Hazara virus to elevated K^+ causes dramatic structural changes in Hazara virus glycoprotein spikes, promotes spike–membrane interactions, and expedites infectivity [61], although the mechanism of this effect needs further study.

3.4.2. Lysosomal Na^+ channels

The lysosome lumen is high in [Na^+], although the ion transporters that establish the lysosomal Na^+ gradient are not yet known [62]. High [Na^+] pretreatment can increase the infectivity of Bunyavirus but not influenza A virus [57,63]. TPCs, which have been shown to be Na^+-selective cation channels in lysosomal electrophysiological analyses [52,64,65], were identified as essential for the release of EBOV's genome into the cytoplasm. Inhibition of TPCs prevents the fusion of EBOV with lysosomes [18], though their activation mechanisms and ion selectivities are areas of active investigation [66,67]. Although lysosomal patch clamp studies have suggested that TPCs are sodium channels, many studies have shown them to be required for NAADP (nicotinic acid adenine dinucleotide phosphate)-mediated Ca^{2+} signaling [52,68, 69]. A recent study showed that the ion selectivity of TPC2 in endo-lysosomes is instead a mutable property that depends on the nature of the activating agonist [70], which may help resolve conflicts in its cation selectivity. Whether Na^+ selectivity of TPC2 is involved in virus invasion still needs further study.

3.5. Lysosomal Ca^{2+}

As the lysosome is an intracellular Ca^{2+} store, with a lumenal [Ca^{2+}] that can reach 0.5 mM, maintained by a putative Ca^{2+}/H^+ exchanger [71]. Ca^{2+} efflux from endosomes and lysosomes is thought to be important for intracellular signaling, organelle acidification and control of many cellular mechanisms including vesicle transport and fusion of lysosomes with late endosomes [72–75]. Calcium sensing proteins including calmodulin and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) have been identified as important for cell entry of EBOV [76], regulated by lysosomal Ca^{2+} flux [77]. NAADP is the most potent of the established Ca^{2+}-mobilizing messengers, and it stimulates intracellular calcium channels to release Ca^{2+} from endosomes and lysosomes [78]. NAADP-induced lysosomal Ca^{2+} flux is required for EBOV [18] and Middle East respiratory syndrome (MERS)-CoV [79]. Thus, compounds that inhibit NAADP-evoked Ca^{2+} release should be studied as potential inhibitors of SARS-CoV-2.

3.5.1. Two-pore channels, Ca^{2+}-permeable channels in the lysosome

TPCs have been shown to be required for NAADP-mediated Ca^{2+} signaling in many studies [69]. TPCs are dimeric ion channels composed of a duplicated domain architecture, and are likely an evolutionary bridge to four-domain voltage-gated Ca^{2+} and Na^+ channels [80–82]. NAADP appears to interact with TPCs indirectly through putative binding proteins [83–86]. In contrast, the endo-lysosomal lipid phosphatidylinositol-3,5-bisphosphate [PI(3,5)P2] has emerged as a direct channel activator that binds within the first domain of TPCs [52, 87,88]. Filoviruses require TPC function for release of the viral genome into the host cell [18], and a pharmacological block of TPCs by trans-Ned-19 (NED19) or other calcium channel blockers inhibits viral infectivity [18].

The exact role of TPCs in viral infection is not clear. Knockdown of TPC1 (biased endosomally) or TPC2 (biased lysosomally) decreased the activity of furin, a protease which facilitates MERS-CoV fusion with cellular membranes [79]. In addition to protease activities, the TPC-specific blocker tetrandrine could prevent the capsid disassembly and nuclear transport required for successful virus entry [89]. The efficacy of tetrandrine is related to the inhibition of viral infection, possibly by preventing viral-endosome membrane fusion from within TPC2-positive structures [18], or by interfering with a TPC2-driven late endosome-lysosome maturation process [20]. Finally, membrane fusion can occur not only between different intracellular compartments, but also between lipid-bound structures such as viral particles and cellular membranes, and they may share common principles [90]. Research has shown that TPC1 is required for formation of contact sites between the ER and endosome, and this was associated with disruptions in late endosome and lysosome morphology [91], which may help explain why TPCs affect virus-endosomal fusion.

A recent study showed that blocking TPC channels by inhibiting PI(3,5)P2 formation led to a pronounced depletion of plasmalemmal Mac-1, which was instead trapped in endomembrane vacuoles [92]. Angiotensin-converting enzyme 2 (ACE2), the primary receptor for SARS-CoV-2, is also a plasmalemmal protein. It is foreseeable that blocking TPC channels could deplete plasmalemmal ACE2 and reduce viral binding, thus affecting the endocytosis of SARS-CoV-2, although this does not seem to be the main reason for TPC's effect on viral infection. Blockage of TPC channels can block virus-endosome membrane fusion and viral gene release to the cytoplasm as detected by a virus contents release assay [18].

3.5.2. TRPML channels, the principal Ca^{2+} release channels in the lysosome

Apart from TPCs, the mucolipin subfamily of transient receptor potential (TRP) cation channels (TRPMLs), which consist of TRPML1, TRPML2 and TRPML3 (a.k.a. MCOLN1–3) are Ca^{2+}-permeable cation channels expressed in the membranes of endosomes and lysosomes [65]. Among these, TRPML2 can increase the infectivity of endocytosed viruses including influenza A virus, yellow fever virus, and Zika by promoting viral vesicular trafficking, resulting in increased endosomal escape [24]. Whether TRPML2 also increases SARS-CoV-2 infection is yet unknown; according to the NextBio Research Disease Atlas (http://www.nextbio.com), a strong downregulation of TRPML3 is associated with cytomegalovirus infection in humans. For TRPML1, although its endogenous agonist PI(3,5)P2 is directly related to the entry of enveloped viruses [93–96], TRPML1 itself does not affect their entry, including SARS-CoV-2 [19,79]. MLSA1, a TRPML1 agonist, also did not affect EBOV infection or rescue EBOV infection in PI(3,5)P2-defective cells [94]. Some studies have shown that rapamycin can increase autophagic flux in a TRPML1-dependent manner [97], and rapamycin's ability to increase the autophagic flux is closely related to viral infectivity [98–100], suggesting that TRPML1 might play an indirect role in antiviral processes.

3.6. Endogenous agonist of TPCs/TRPMLs: PI(3,5)P2

PI(3,5)P2 is a late endosome/lysosome-specific phosphoinositide, produced by the lipid kinase PIKfyve in mammalian cells [91]. PI(3,5)P2 regulates membrane trafficking and activity of ion channels [91], and its effectors are fewer compared to those of Phosphatidylinositol 3-phosphate (PtdIns3P). The scarce effectors of PI(3,5)P2 thus far identified include TRPMLs and TPCs [52,101], all of which are directly or indirectly related to the entry of enveloped viruses.

Viruses that require deeper trafficking in the endocytic pathway to endo-lysosomes rely on PI(3,5)P2. Inhibition of PIKfyve and PI(3,5)P2 production by apilimod or YM201636 has been shown to inhibit viral

fusion in late endosomes and lysosomes, for example in vaccinia virus [96], African swine fever virus [102], SARS-CoV-2 [19,103], and some filoviruses [93,94]. The mechanism of inhibition is likely due to a defect in the maturation of endo-lysosomes, impairing traffic of incoming virus to sites where NPC1 resides and membrane fusion takes place [93].

4. Open questions and future challenges

Viruses have developed strategies to exploit host cell machinery and organelles to promote viral infection. Experiments have shown that entry of SARS-CoV-2 into host cells is mainly mediated by endocytosis, and is closely related to lysosomes [19,22,104,105]. More specifically, TPC2 is critical for SARS-CoV-2 infection, which supports the notion that lysosomal ion channels may be targets for therapeutic strategies against SARS-CoV-2. Lysosomes are equipped with various ion channels, some of which have been proven to affect entry of specific enveloped viruses (see the summary in Table 1). There are many licensed pharmaceutical preparations that target ion channels, improving our understanding of the role of ion channels in viral pathogenesis may reveal their potential as targets of new, safe drugs with broad antiviral activity.

Although several lysosomal ion channels have been confirmed as potential novel targets for the treatment of viruses, several basic questions remain:

- What is the role of TPCs in viral uncoating?
- How does NAADP induce calcium release from lysosomes, and what is the role for the NAADP-dependent Ca^{2+} signaling in viral translocation?
- How do lysosomal $\Delta\psi$ and Ca^{2+} regulate the process of membrane fusion between enveloped viruses and lysosomes?
- Are there any other (known or unknown) lysosomal ion channel that may affect viral entry?

Further investigation is required to address these questions, which will help us gain a much better understanding of the functional relevance of these lysosomal ion channels. In addition, these investigations may discover potential new therapeutic lines in the fight against SARS-CoV-2.

Declaration of Competing Interest

The authors declare no conflict of interests.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (32041003 and 31872488). The professional editing service NB Revisions was used for technical preparation of the text prior to submission.

References

[1] F. Li, Structure, function, and evolution of coronavirus spike proteins, Annu. Rev. Virol. 3 (2016) 237–261.
[2] R.J. Hulswit, C.A. de Haan, B.J. Bosch, Coronavirus spike protein and tropism changes, Adv. Virus Res. 96 (2016) 29–57.
[3] J.E. Lee, E.O. Saphire, Ebolavirus glycoprotein structure and mechanism of entry, Future Virol. 4 (2009) 621–635.
[4] S. Nagashima, M. Takahashi, T. Kobayashi, Tanggis, T. T. Nishizawa, P. P. Nishiyama, Primadharsini, H. Okamoto, Characterization of the quasi-enveloped hepatitis E virus particles released by the cellular exosomal pathway, J. Virol. 91 (2017).
[5] Z. Feng, L. Hensley, K.L. McKnight, F. Hu, V. Madden, L. Ping, S.H. Jeong, C. Walker, R.E. Lanford, S.M. Lemon, A pathogenic picornavirus acquires an envelope by hijacking cellular membranes, Nature 496 (2013) 367–371.
[6] E.E. Rivera-Serrano, O. Gonzalez-Lopez, A. Das, S.M. Lemon, Cellular entry and uncoating of naked and quasi-enveloped human hepatoviruses, Elife 8 (2019).
[7] J. Zhang, J. Chen, D. Shi, H. Shi, X. Zhang, J. Liu, L. Cao, X. Zhu, Y. Liu, X. Wang, Z. Ji, L. Feng, Porcine deltacoronavirus enters cells via two pathways: a protease-mediated one at the cell surface and another facilitated by cathepsins in the endosome, J. Biol. Chem. 294 (2019) 9830–9843.
[8] Y.L. Yang, F. Meng, P. Qin, G. Herrler, Y.W. Huang, Y.D. Tang, Trypsin promotes porcine deltacoronavirus mediating cell-to-cell fusion in a cell type-dependent manner, Emerg. Microbes Infect. 9 (2020) 457–468.
[9] E. Sun, J. He, X. Zhuang, Live cell imaging of viral entry, Curr. Opin. Virol. 3 (2013) 34–43.
[10] M. Ehrlich, W. Boll, A. Van Oijen, R. Hariharan, K. Chandran, M.L. Nibert, T. Kirchhausen, Endocytosis by random initiation and stabilization of clathrin-coated pits, Cell 118 (2004) 591–605.
[11] A. Nanbo, M. Imai, S. Watanabe, T. Noda, K. Takahashi, G. Neumann, P. Halfmann, Y. Kawaoka, Ebolavirus is internalized into host cells via macropinocytosis in a viral glycoprotein-dependent manner, PLoS Pathog. 6 (2010), e1001121.
[12] M.F. Saeed, A.A. Kolokoltsov, T. Albrecht, R.A. Davey, Cellular entry of ebola virus involves uptake by a macropinocytosis-like mechanism and subsequent trafficking through early and late endosomes, PLoS Pathog. 6 (2010), e1001110.
[13] J. Mercer, M. Schelhaas, A. Helenius, Virus entry by endocytosis, Annu. Rev. Biochem. 79 (2010) 803–833.
[14] M. Cote, J. Misasi, T. Ren, A. Bruchez, K. Lee, C.M. Filone, L. Hensley, Q. Li, D. Ory, K. Chandran, J. Cunningham, Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection, Nature 477 (2011) 344–348.
[15] J.E. Carette, M. Raaben, A.C. Wong, A.S. Herbert, G. Obernosterer, N. Mulherkar, A.I. Kuehne, P.J. Kranzusch, A.M. Griffin, G. Ruthel, P. Dal Cin, J.M. Dye, S. P. Whelan, K. Chandran, T.R. Brummelkamp, Ebola virus entry requires the cholesterol transporter Niemann-Pick C1, Nature 477 (2011) 340–343.
[16] E.H. Miller, G. Obernosterer, M. Raaben, A.S. Herbert, M.S. Deffieu, A. Krishnan, E. Ndungo, R.G. Sandesara, J.E. Carette, A.I. Kuehne, G. Ruthel, S.R. Pfeffer, J. M. Dye, S.P. Whelan, T.R. Brummelkamp, K. Chandran, Ebola virus entry requires the host-programmed recognition of an intracellular receptor, EMBO J. 31 (2012) 1947–1960.
[17] L.T. Jae, M. Raaben, A.S. Herbert, A.I. Kuehne, A.S. Wirchnianski, T.K. Soh, S. H. Stubbs, H. Janssen, M. Damme, P. Saftig, S.P. Whelan, J.M. Dye, T. R. Brummelkamp, Virus entry. Lassa virus entry requires a trigger-induced receptor switch, Science 344 (2014) 1506–1510.
[18] Y. Sakurai, A.A. Kolokoltsov, C.C. Chen, M.W. Tidwell, W.E. Bauta, N. Klugbauer, C. Grimm, C. Wahl-Schott, M. Biel, R.A. Davey, Ebola virus. Two-pore channels control Ebola virus host cell entry and are drug targets for disease treatment, Science 347 (2015) 995–998.
[19] X. Ou, Y. Liu, X. Lei, P. Li, D. Mi, L. Ren, L. Guo, R. Guo, T. Chen, J. Hu, Z. Xiang, Z. Mu, X. Chen, J. Chen, K. Hu, Q. Jin, J. Wang, Z. Qian, Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV, Nat. Commun. 11 (2020) 1620.
[20] J.A. Simmons, R.S. D'Souza, M. Ruas, A. Galione, J.E. Casanova, J.M. White, Ebolavirus glycoprotein directs fusion through NPC1+ endolysosomes, J. Virol. 90 (2016) 605–610.
[21] C. Grimm, R. Tang, Could an endo-lysosomal ion channel be the Achilles heel of SARS-CoV2? Cell Calcium 88 (2020), 102212.
[22] A. Filippini, A. D'Amore, F. Palombi, A. Carpaneto, Could the inhibition of endo-lysosomal two-pore channels (TPCs) by the natural flavonoid naringenin represent an option to Fight SARS-CoV-2 Infection? Front. Microbiol. 11 (2020) 970.
[23] C.J. Penny, K. Vassileva, A. Jha, Y. Yuan, X. Chee, E. Yates, M. Mazzon, B. S. Kilpatrick, S. Muallem, M. Marsh, T. Rahman, S. Patel, Mining of Ebola virus entry inhibitors identifies approved drugs as two-pore channel pore blockers, Biochim. Biophys. Acta Mol. Cell Res. 1866 (2019) 1151–1161.
[24] N. Rinkenberger, J.W. Schoggins, Mucolipin-2 cation channel increases trafficking efficiency of endocytosed viruses, Mbio 9 (2018).
[25] T. Kolter, K. Sandhoff, Principles of lysosomal membrane digestion: stimulation of sphingolipid degradation by sphingolipid activator proteins and anionic lysosomal lipids, Annu. Rev. Cell Dev. Biol. 21 (2005) 81–103.
[26] R.M. Perera, R. Zoncu, The lysosome as a regulatory hub, Annu. Rev. Cell Dev. Biol. 32 (2016) 223–253.
[27] C.Y. Lim, R. Zoncu, The lysosome as a command-and-control center for cellular metabolism, J. Cell Biol. 214 (2016) 653–664.
[28] A. Efeyan, R. Zoncu, D.M. Sabatini, Amino acids and mTORC1: from lysosomes to disease, Trends Mol. Med. 18 (2012) 524–533.
[29] H. Xu, D. Ren, Lysosomal physiology, Annu. Rev. Physiol. 77 (2015) 57–80.
[30] C.C. Scott, J. Gruenberg, Ion flux and the function of endosomes and lysosomes: pH is just the start: the flux of ions across endosomal membranes influences endosome function not only through regulation of the luminal pH, Bioessays 33 (2011) 103–110.
[31] X.P. Dong, X. Cheng, E. Mills, M. Delling, F. Wang, T. Kurz, H. Xu, The type IV mucolipidosis-associated protein TRPML1 is an endolysosomal iron release channel, Nature 455 (2008) 992–996.
[32] X.P. Dong, X. Wang, D. Shen, S. Chen, M. Liu, Y. Wang, E. Mills, X. Cheng, M. Delling, H. Xu, Activating mutations of the TRPML1 channel revealed by proline-scanning mutagenesis, J. Biol. Chem. 284 (2009) 32040–32052.
[33] C.L. Cang, B. Bekele, D.J. Ren, The voltage-gated sodium channel TPC1 confers endolysosomal excitability, Nat. Chem. Biol. 10 (2014) 463–469.
[34] D.E. Johnson, P. Ostrowski, V. Jaumouille, S. Grinstein, The position of lysosomes within the cell determines their luminal pH, J. Cell Biol. 212 (2016) 677–692.
[35] R. Konig, S. Stertz, Y. Zhou, A. Inoue, H.H. Hoffmann, S. Bhattacharyya, J. G. Alamares, D.M. Tscherne, M.B. Ortigoza, Y. Liang, Q. Gao, S.E. Andrews, S. Bandyopadhyay, P. De Jesus, B.P. Tu, L. Pache, C. Shih, A. Orth, G. Bonamy, L. Miraglia, T. Ideker, A. Garcia-Sastre, J.A. Young, P. Palese, M.L. Shaw, S.

K. Chanda, Human host factors required for influenza virus replication, Nature 463 (2010) 813–817.

[36] R. Guinea, L. Carrasco, Requirement for vacuolar proton-ATPase activity during entry of influenza virus into cells, J. Virol. 69 (1995) 2306–2312.

[37] M. Degtyarev, A. De Maziere, C. Orr, J. Lin, B.B. Lee, J.Y. Tien, W.W. Prior, S. van Dijk, H. Wu, D.C. Gray, D.P. Davis, H.M. Stern, L.J. Murray, K.P. Hoeflich, J. Klumperman, L.S. Friedman, K. Lin, Akt inhibition promotes autophagy and sensitizes PTEN-null tumors to lysosomotropic agents, J. Cell Biol. 183 (2008) 101–116.

[38] A. Savarino, L. Di Trani, I. Donatelli, R. Cauda, A. Cassone, New insights into the antiviral effects of chloroquine, Lancet Infect. Dis. 6 (2006) 67–69.

[39] M.J. Vincent, E. Bergeron, S. Benjannet, B.R. Erickson, P.E. Rollin, T.G. Ksiazek, N.G. Seidah, S.T. Nichol, Chloroquine is a potent inhibitor of SARS coronavirus infection and spread, Virol. J. 2 (2005) 69.

[40] H. Wang, P. Yang, K. Liu, F. Guo, Y. Zhang, G. Zhang, C. Jiang, SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway, Cell Res. 18 (2008) 290–301.

[41] G. Simmons, J.D. Reeves, A.J. Rennekamp, S.M. Amberg, A.J. Piefer, P. Bates, Characterization of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) spike glycoprotein-mediated viral entry, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 4240–4245.

[42] S.M. Gregory, E. Harada, B. Liang, S.E. Delos, J.M. White, L.K. Tamm, Structure and function of the complete internal fusion loop from Ebolavirus glycoprotein 2, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 11211–11216.

[43] S.M. Gregory, P. Larsson, E.A. Nelson, P.M. Kasson, J.M. White, L.K. Tamm, Ebolavirus entry requires a compact hydrophobic fist at the tip of the fusion loop, J. Virol. 88 (2014) 6636–6649.

[44] P.Y. Lozach, J. Huotari, A. Helenius, Late-penetrating viruses, Curr. Opin. Virol. 1 (2011) 35–43.

[45] K. Chakraborty, K. Leung, Y. Krishnan, High lumenal chloride in the lysosome is critical for lysosome function, Elife 6 (2017).

[46] A.R. Graves, P.K. Curran, C.L. Smith, J.A. Mindell, The Cl-/H+ antiporter ClC-7 is the primary chloride permeation pathway in lysosomes, Nature 453 (2008) 788–792.

[47] T.J. Jentsch, M. Pusch, CLC chloride channels and transporters: structure, function, physiology, and disease, Physiol. Rev. 98 (2018) 1493–1590.

[48] K. Zheng, M. Chen, Y. Xiang, K. Ma, F. Jin, X. Wang, X. Wang, S. Wang, Y. Wang, Inhibition of herpes simplex virus type 1 entry by chloride channel inhibitors tamoxifen and NPPB, Biochem. Biophys. Res. Commun. 446 (2014) 990–996.

[49] Z. Igloi, B.P. Mohl, J.D. Lippiat, M. Harris, J. Mankouri, Requirement for chloride channel function during the hepatitis C virus life cycle, J. Virol. 89 (2015) 4023–4029.

[50] A.J. Morgan, F.M. Platt, E. Lloyd-Evans, A. Galione, Molecular mechanisms of endolysosomal Ca2+ signalling in health and disease, Biochem. J. 439 (2011) 349–374.

[51] B.E. Steinberg, K.K. Huynh, A. Brodovitch, S. Jabs, T. Stauber, T.J. Jentsch, S. Grinstein, A cation counterflux supports lysosomal acidification, J. Cell Biol. 189 (2010) 1171–1186.

[52] X. Wang, X.L. Zhang, X.P. Dong, M. Samie, X.R. Li, X.P. Cheng, A. Goschka, D. B. Shen, Y.D. Zhou, J. Harlow, M.X. Zhu, D.E. Clapham, D.J. Ren, H.X. Xu, TPC proteins are phosphoinositide-activated sodium-selective ion channels in endosomes and lysosomes, Cell 151 (2012) 372–383.

[53] W. Wang, X. Zhang, Q. Gao, M. Lawas, L. Yu, X. Cheng, M. Gu, N. Sahoo, X. Li, P. Li, S. Ireland, A. Meredith, H. Xu, A voltage-dependent K(+) channel in the lysosome is required for refilling lysosomal Ca(2+) stores, J. Cell Biol. 216 (2017) 1715–1730.

[54] C. Cang, K. Aranda, Y.J. Seo, B. Gasnier, D. Ren, TMEM175 is an organelle K(+) channel regulating lysosomal function, Cell 162 (2015) 1101–1112.

[55] Q. Cao, X.Z. Zhong, Y. Zou, Z. Zhang, L. Toro, X.P. Dong, BK channels alleviate lysosomal storage diseases by providing positive feedback regulation of lysosomal Ca2+ release, Dev. Cell 33 (2015) 427–441.

[56] G.A. Wyant, M. Abu-Remaileh, R.L. Wolfson, W.W. Chen, E. Freinkman, L. V. Danai, M.G. Vander Heiden, D.M. Sabatini, mTORC1 activator SLC38A9 is required to efflux essential amino acids from lysosomes and use protein as a nutrient, Cell 171 (2017) 642–654, e612.

[57] S. Hover, B. Foster, J. Fontana, A. Kohl, S.A.N. Goldstein, J.N. Barr, J. Mankouri, Bunyavirus requirement for endosomal K+ reveals new roles of cellular ion channels during infection, PLoS Pathog. 14 (2018), e1006845.

[58] X. Feng, Z. Zhao, Q. Li, Z. Tan, Lysosomal potassium channels: potential roles in lysosomal function and neurodegenerative diseases, CNS Neurol. Disord. Drug Targets 17 (2018) 261–266.

[59] S. Hover, B. King, B. Hall, E.A. Loundras, H. Taqi, J. Daly, M. Dallas, C. Peers, E. Schnettler, C. McKimmie, A. Kohl, J.N. Barr, J. Mankouri, Modulation of potassium channels inhibits bunyavirus infection, J. Biol. Chem. 291 (2016) 3411–3422.

[60] N. Bobak, S. Feliciangeli, C.C. Chen, I. Ben Soussia, S. Bittner, S. Pagnotta, T. Ruck, M. Biel, C. Wahl-Schott, C. Grimm, S.G. Meuth, F. Lesage, Recombinant tandem of pore-domains in a weakly inward rectifying K(+) channel 2 (TWIK2) forms active lysosomal channels, Sci. Rep. 7 (2017) 649.

[61] E.K. Punch, S. Hover, H.T.W. Blest, J. Fuller, R. Hewson, J. Fontana, J. Mankouri, J.N. Barr, Potassium is a trigger for conformational change in the fusion spike of an enveloped RNA virus, J. Biol. Chem. 293 (2018) 9937–9944.

[62] P. Li, M. Gu, H. Xu, Lysosomal ion channels as decoders of cellular signals, Trends Biochem. Sci. 44 (2019) 110–124.

[63] S. Stauffer, Y. Feng, F. Nebioglu, R. Heilig, P. Picotti, A. Helenius, Stepwise priming by acidic pH and a high K+ concentration is required for efficient uncoating of influenza A virus cores after penetration, J. Virol. 88 (2014) 13029–13046.

[64] C. Grimm, E. Butz, C.C. Chen, C. Wahl-Schott, M. Biel, From mucolipidosis type IV to Ebola: TRPML and two-pore channels at the crossroads of endo-lysosomal trafficking and disease, Cell Calcium 67 (2017) 148–155.

[65] H.Xa.D. Ren, Lysosomal physiology, Annu. Rev. Physiol. (2015).

[66] J.S. Marchant, S. Patel, Questioning regulation of two-pore channels by NAADP, Messenger (Los Angel) 2 (2013) 113–119.

[67] A.J. Morgan, A. Galione, Two-pore channels (TPCs): current controversies, Bioessays 36 (2014) 173–183.

[68] C. Cang, Y. Zhou, B. Navarro, Y.J. Seo, K. Aranda, L. Shi, S. Battaglia-Hsu, I. Nissim, D.E. Clapham, D. Ren, mTOR regulates lysosomal ATP-sensitive two-pore Na(+) channels to adapt to metabolic state, Cell 152 (2013) 778–790.

[69] P.J. Calcraft, M. Ruas, Z. Pan, X. Cheng, A. Arredouani, X. Hao, J. Tang, K. Rietdorf, L. Teboul, K.T. Chuang, P. Lin, R. Xiao, C. Wang, Y. Zhu, Y. Lin, C. N. Wyatt, J. Parrington, J. Ma, A.M. Evans, A. Galione, M.X. Zhu, NAADP mobilizes calcium from acidic organelles through two-pore channels, Nature 459 (2009) 596–600.

[70] S. Gerndt, C.C. Chen, Y.K. Chao, Y. Yuan, S. Burgstaller, A. Scotto Rosato, E. Krogsaeter, N. Urban, K. Jacob, O.N.P. Nguyen, M.T. Miller, M. Keller, A. M. Vollmar, T. Gudermann, S. Zierler, J. Schredelseker, M. Schaefer, M. Biel, R. Malli, C. Wahl-Schott, F. Bracher, S. Patel, C. Grimm, Agonist-mediated switching of ion selectivity in TPC2 differentially promotes lysosomal function, Elife 9 (2020).

[71] J. Yang, Z. Zhao, M. Gu, X. Feng, H. Xu, Release and uptake mechanisms of vesicular Ca(2+) stores, Protein Cell 10 (2019) 8–19.

[72] J.C. Hay, Calcium: a fundamental regulator of intracellular membrane fusion? EMBO Rep. 8 (2007) 236–240.

[73] J.P. Luzio, N.A. Bright, P.R. Pryor, The role of calcium and other ions in sorting and delivery in the late endocytic pathway, Biochem. Soc. Trans. 35 (2007) 1088–1091.

[74] X. Li, A.G. Garrity, H. Xu, Regulation of membrane trafficking by signalling on endosomal and lysosomal membranes, J Physiol 591 (2013) 4389–4401.

[75] J.A. Mindell, Lysosomal acidification mechanisms, Annu. Rev. Physiol. 74 (2012) 69–86.

[76] A.A. Kolokoltsov, M.F. Saeed, A.N. Freiberg, M.R. Holbrook, R.A. Davey, Identification of novel cellular targets for therapeutic intervention against Ebola virus infection by siRNA screening, Drug Dev. Res. 70 (2009) 255–265.

[77] D.L. Medina, A. Ballabio, Lysosomal calcium regulates autophagy, Autophagy 11 (2015) 970–971.

[78] A. Galione, NAADP receptors, Cold Spring Harb. Perspect. Biol. 11 (2019).

[79] G.S. Gunaratne, Y. Yang, F. Li, T.F. Walseth, J.S. Marchant, NAADP-dependent Ca (2+) signaling regulates Middle East respiratory syndrome-coronavirus pseudovirus translocation through the endolysosomal system, Cell Calcium 75 (2018) 30–41.

[80] D. Churamani, R. Hooper, E. Brailoiu, S. Patel, Domain assembly of NAADP-gated two-pore channels, Biochem. J. 441 (2012) 317–323.

[81] T. Rahman, X. Cai, G.C. Brailoiu, M.E. Abood, E. Brailoiu, S. Patel, Two-pore channels provide insight into the evolution of voltage-gated Ca2+ and Na+ channels, Sci. Signal. 7 (2014) ra109.

[82] C.J. Penny, T. Rahman, A. Sula, A.J. Miles, B.A. Wallace, S. Patel, Isolated pores dissected from human two-pore channel 2 are functional, Sci. Rep. 6 (2016) 38426.

[83] Y. Lin-Moshier, T.F. Walseth, D. Churamani, S.M. Davidson, J.T. Slama, R. Hooper, E. Brailoiu, S. Patel, J.S. Marchant, Photoaffinity labeling of nicotinic acid adenine dinucleotide phosphate (NAADP) targets in mammalian cells, J. Biol. Chem. 287 (2012) 2296–2307.

[84] T.F. Walseth, Y. Lin-Moshier, P. Jain, M. Ruas, J. Parrington, A. Galione, J. S. Marchant, J.T. Slama, Photoaffinity labeling of high affinity nicotinic acid adenine dinucleotide phosphate (NAADP)-binding proteins in sea urchin egg, J. Biol. Chem. 287 (2012) 2308–2315.

[85] T.F. Walseth, Y. Lin-Moshier, K. Weber, J.S. Marchant, J.T. Slama, A.H. Guse, Nicotinic acid adenine dinucleotide 2'-phosphate (NAADP) binding proteins in T-lymphocytes, Messenger (Los Angel) 1 (2012) 86–94.

[86] J.S. Marchant, Y. Lin-Moshier, T.F. Walseth, S. Patel, The molecular basis for Ca(2+) signalling by NAADP: two-pore channels in a complex? Messenger (Los Angel) 1 (2012) 63–76.

[87] J. She, J. Guo, Q. Chen, W. Zeng, Y. Jiang, X.C. Bai, Structural insights into the voltage and phospholipid activation of the mammalian TPC1 channel, Nature 556 (2018) 130–134.

[88] S. Patel, Two-pore channels open up, Nature 556 (2018) 38–40.

[89] S.J. Dobson, J. Mankouri, A. Whitehouse, Identification of potassium and calcium channel inhibitors as modulators of polyomavirus endosomal trafficking, Antiviral Res. (2020), 104819.

[90] S. Martens, H.T. McMahon, Mechanisms of membrane fusion: disparate players and common principles, Nat. Rev. Mol. Cell Biol. 9 (2008) 543–556.

[91] B.S. Kilpatrick, E.R. Eden, L.N. Hockey, E. Yates, C.E. Futter, S. Patel, An endosomal NAADP-Sensitive two-pore Ca(2+) channel regulates ER-endosome membrane contact sites to control growth factor signaling, Cell Rep. 18 (2017) 1636–1645.

[92] S.A. Freeman, S. Uderhardt, A. Saric, R.F. Collins, C.M. Buckley, S. Mylvaganam, P. Boroumand, J. Plumb, R.N. Germain, D. Ren, S. Grinstein, Lipid-gated monovalent ion fluxes regulate endocytic traffic and support immune surveillance, Science (2019).

[93] E.A. Nelson, J. Dyall, T. Hoenen, A.B. Barnes, H. Zhou, J.Y. Liang, J. Michelotti, W.H. Dewey, L.E. DeWald, R.S. Bennett, P.J. Morris, R. Guha, C. Klumpp-Thomas,

C. McKnight, Y.C. Chen, X. Xu, A. Wang, E. Hughes, S. Martin, C. Thomas, P. B. Jahrling, L.E. Hensley, G.G. Olinger Jr., J.M. White, The phosphatidylinositol-3-phosphate 5-kinase inhibitor apilimod blocks filoviral entry and infection, PLoS Negl. Trop. Dis. 11 (2017), e0005540.

[94] S. Qiu, A. Leung, Y. Bo, R.A. Kozak, S.P. Anand, C. Warkentin, F.D.R. Salambanga, J. Cui, G. Kobinger, D. Kobasa, M. Cote, Ebola virus requires phosphatidylinositol (3,5) bisphosphate production for efficient viral entry, Virology 513 (2018) 17–28.

[95] M.A. Cuesta-Geijo, L. Barrado-Gil, I. Galindo, R. Munoz-Moreno, C. Alonso, Redistribution of endosomal membranes to the African swine fever virus replication site, Viruses 9 (2017).

[96] Z. Rizopoulos, G. Balistreri, S. Kilcher, C.K. Martin, M. Syedbasha, A. Helenius, J. Mercer, Vaccinia virus infection requires maturation of Macropinosomes, Traffic 16 (2015) 814–831.

[97] X. Zhang, W. Chen, Q. Gao, J. Yang, X. Yan, H. Zhao, L. Su, M. Yang, C. Gao, Y. Yao, K. Inoki, D. Li, R. Shao, S. Wang, N. Sahoo, F. Kudo, T. Eguchi, B. Ruan, H. Xu, Rapamycin directly activates lysosomal mucolipin TRP channels independent of mTOR, PLoS Biol. 17 (2019), e3000252.

[98] S. Ko, M.J. Gu, C.G. Kim, Y.C. Kye, Y. Lim, J.E. Lee, B.C. Park, H. Chu, S.H. Han, C.H. Yun, Rapamycin-induced autophagy restricts porcine epidemic diarrhea virus infectivity in porcine intestinal epithelial cells, Antiviral Res. 146 (2017) 86–95.

[99] Y. Choi, J.W. Bowman, J.U. Jung, Autophagy during viral infection - a double-edged sword, Nat. Rev. Microbiol. 16 (2018) 341–354.

[100] J. Mao, E. Lin, L. He, J. Yu, P. Tan, Y. Zhou, Autophagy and viral infection, Adv. Exp. Med. Biol. 1209 (2019) 55–78.

[101] X.P. Dong, D. Shen, X. Wang, T. Dawson, X. Li, Q. Zhang, X. Cheng, Y. Zhang, L. S. Weisman, M. Delling, H. Xu, PI(3,5)P(2) controls membrane trafficking by direct activation of mucolipin Ca(2+) release channels in the endolysosome, Nat. Commun. 1 (2010) 38.

[102] M.A. Cuesta-Geijo, I. Galindo, B. Hernaez, J.I. Quetglas, I. Dalmau-Mena, C. Alonso, Endosomal maturation, Rab7 GTPase and phosphoinositides in African swine fever virus entry, PLoS One 7 (2012), e48853.

[103] L. Riva, S. Yuan, X. Yin, L. Martin-Sancho, N. Matsunaga, S. Burgstaller-Muehlbacher, L. Pache, P.P. De Jesus, M.V. Hull, M. Chang, J.F. Chan, J. Cao, V. K. Poon, K. Herbert, T.T. Nguyen, Y. Pu, C. Nguyen, A. Rubanov, L. Martinez-Sobrido, W.C. Liu, L. Miorin, K.M. White, J.R. Johnson, C. Benner, R. Sun, P. G. Schultz, A. Su, A. Garcia-Sastre, A.K. Chatterjee, K.Y. Yuen, S.K. Chanda, A large-scale drug repositioning survey for SARS-CoV-2 antivirals, bioRxiv (2020).

[104] M. Wang, R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, Z. Shi, Z. Hu, W. Zhong, G. Xiao, Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro, Cell Res. 30 (2020) 269–271.

[105] R.A. Ballout, D. Sviridov, M.I. Bukrinsky, A.T. Remaley, The lysosome: a potential juncture between SARS-CoV-2 infectivity and Niemann-Pick disease type C, with therapeutic implications, FASEB J. (2020).