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Review

Lipidomics issues on human positive ssRNA virus infection: an update

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Abstract: Recent COVID-19 outbreak has come into prominence the pathogenetic mechanisms underlying the Biology and Biochemistry of viral infections. COVID-19 illness is brought about by infection with the severe acute respiratory syndrome coronavirus SARS-CoV-2 [1,2], an enveloped positive single stranded RNA virus (ssRNA+). From a lipidomics viewpoint, there is a variety of mechanisms involving virus infection that encompass virus entry, disturbance of host cell lipid metabolism, and the role played by diverse lipids in regard to the infection effectiveness. All these aspects have currently been tackled separately as independent issues and focusing on the function of proteins. Here we review the role of cholesterol and other lipids in in ssRNA+ and SARS-COV-2 infection.

Keywords: Lipidomics, ssRNA+ virus, membrane fusion, lipid metabolism, cholesterol, sphingolipids, phosphatidylinositol

1. Introduction

Ongoing COVID-19 pandemic develops (July 2020) with devastating global consequences, both for social organization and healthcare system. COVID-19 illness is brought about by infection with the severe acute respiratory syndrome coronavirus SARS-CoV-2 [1,2], which is an enveloped positive single stranded RNA virus (ssRNA+) [3]. The most abundant studies related to human diseases induced by ssRNA positive virus referred to *Picornaviridae*, *Coronaviridae* and *Flaviviridae* [4].

This impact in a short time span has brought the Biology and Biochemistry of viral infection mechanisms to reach momentum. The infection mechanisms have been described for other unrelated viral families [5]. Most of them are DNA viruses and belong to groups I or II according to he Baltimore's classification. Within Picornaviridae, Coronaviridae and Flaviviridae, the most studied virus are Rhino and Poliovirus (Picornaviridae), SARS-CoV, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Hepatitis C virus (HCV), West Nile virus (WNV) and Dengue virus (DENV). Nonetheless, some aspects regarding the influenza virus from the Orthomysoviridae family, and the human immunodeficiency virus from the Retroviridae family, will be considered as major knowledge that has been gained from the biological point of view as it is the virus entry mechanism.

All ssRNA+ viruses belong to the Group IV in the classification of Baltimore [6]. They initially infect mammal cells through the interaction of virus proteins with any given host cell protein. Further fusion of the virus and host cell membranes is required for the viral genetic material to get into the cell. Once inside the cell, the genomic and subgenomic viral RNAs are translated into the virus proteins, which lead the virus replication, a process that involves modulation of the host cell lipid metabolism [3,5,7]. Consequently, along with other features, current lipid studies about

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SARS-CoV-2 infection focus their research on membrane fusion and modulation of the lipid metabolism of the host cell. These two processes are considered separated disciplines of the infection.

The fight against the virus infection encompasses primarily the inhibition of the binding of the viral spike protein to the host cell receptor protein. Consequently, most of current research focuses on the role played by viral proteins and the lipid environment but regulation is being considered secondarily [8]. Nevertheless, improving the knowledge on how the lipids are involved in the mechanisms of infection may provide clues to develop treatments and better counteract the virus-induced pathology [3]. To fill this gap, here we review the main aspects regarding the lipidome regulation of the viral infection mechanism by ssRNA+ viruses, with special focus on SARS-CoV-2.

2. Virus entry: lipid rafts and membrane domains

2.1. Membrane mechanical properties required for virus infection

The initial step in virus infection is binding of any viral structural glycoprotein to a receptor of the host cell. The spike protein accounts for such function in coronaviruses (CoVs) and other enveloped viruses. After the virus is attached to the host cell protein, the process of membrane fusion starts to get the viral genome into the host cell. This process implies viral envelope and host cell membrane fusion, for which an energetically cost-effective barrier should be overcome. Membrane fusion is driven by the fusion peptide (class I) in coronaviruses, which is localized within the spike protein (S protein) and becomes active after cleavage of the S protein at specific sites by host proteases **[4,7,9]**. A different mechanism of attachment and endocytosis drives the virus entry in the case of HCV. This mechanism is more complex than that of coronaviruses and involves interaction of the virus envelope E1 and E2 proteins (class II fusion loop) with several host cell proteins **[10–12]**. However, a membrane fusion-driven pore is also required in HCV to deliver the viral genetic material into the host cell cytoplasm.

Two main mechanisms of membrane fusion have been described: viral endocytosis by host cell membrane (endocytic pathway), and both viral and host cell membrane fusion (non-endocytic pathway). Membrane fusion has been described to proceed through the catalytic action of three different types of fusion peptides or fusion loops of class I, II or III. These proteins afford the free energy necessary to overcome the kinetic barrier due to repulsive hydration strength through conformational changes. Most of the knowledge on the viral and host membrane fusion has been gained from the influenza virus and its type I fusion peptide hemagglutinin. Detailed description of the three fusion peptide-guided mechanisms involved in membrane fusion has been previously reviewed in **[13–15]**. Bringing the viral and the host membranes into the required proximity (c.a. 20 Å) for inducing the membrane fusion is a process that entails membrane curvature and changes in the lipid bilayer phase. They are accounted for by the insertion of a hydrophobic region of the fusion peptide, which requires dehydration of the inter-membrane space. Nonetheless, from experiments with no-protein fusogens, like polyethilen glycol, it seems that membrane curvature stabilization is not a key player in membrane pore opening. The calculated displacement of lipids in the outer leaflet of the host membrane accounts for no more than 10% of the membrane area (about 3500 $Å^2$), which does not represent a substantial energetic demand **[14]**. This energetic burden has been demonstrated to be afforded by the cooperation of three fusion peptides in influenza virus membrane fusion **[16]**; whereas two adjacent trimers of the fusion protein have been found to be required in West Nile virus **[17]**. This fact points to the viral membrane curvature may not impose constrain for proceeding to the hemifusion step with the formation of a steep curvature stalk, where the outer leafleats are merged. By mesurement of electron density profiles through X-Ray reflectivity in stalks formed from bilayers in a lamellar state with different lipid compositions, Aeffner et al. **[18]** determined that the inter-bilayer separation should attain 9.0±0.5 Å in order to facilitate dehydration and promote stalk formation. These authors also found that increasing the relative proportion of

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nonbilayer-forming, cone-shaped lipids, like glycerophosphoethanolamine or cholesterol, favored the stalk formation by reducing the hydration energy barrier and, possibly, by contributing with their intrinsic negative curvature. As well, the energy required for dehydration was, in this study, found to decrease with the length of the acyl chains in the glycerophospholipids. The hemifusion stalk stage was, however, not detected by Gui et al. **[19]** using fluorescence and electron microscopy. The results of this study show that such stage might be an unstable intermediate that is quickly resolved towards the postfusion stage. Contrarily, localized point-like contacts were abundantly visualized in this study, where the dimples formed in the target membrane, about 5 nm wide, were drawn towards the virus surface. They were able to detect up to well-resolved four types of virus-target membranes contacts at pH 5.5 and 5.25 using liposomes of dioleylglycerylphosphatidylcholine, DOPC, with 20% cholesterol. At the lowest pH, a tightly contact of the two membranes through an extended length of about 100 nm (catalogued by the authors as type III) was the predominant interaction, whose abundance was increased by about 3-fold in cholesterol-containing liposomes in comparison to only DOPC liposomes.

Using synthetic peptides that resemble the hemagglutinin fusion peptide and electron spin resonance (ESR), Ge and Freed **[20]** found that the most relevant effect of synthetic fusion peptide was the induction of highly ordered membrane domains, which came motivated by virtue of electrostatic interactions between the peptide and negatively charged phospholipid headgroups. A similar effect was reported for two putative fusion peptides enclosed in the spike glycoprotein of SARS-CoV-1. It was found in this study that the inner water content in the lipid bilayer was dropped by the insertion of the fusion peptide as a consequence of increased lipid packing, but only in membranes containing negatively charged lipids, whereas only slightly altered in zwitterionic dipalmitoylglycerophosphatidylcholine (DPPC) liposomes **[21]**. Additionally, the fusion peptides created opposing curvature stresses in the highly bended membranes containing nonbilayer-forming phospholipids. However, previous studies had pointed out that interaction with the lipid headgroups was not an essential factor in reaching the membrane hemifusion state **[14,22]**. In SARS-CoV, the possibility of existing two fusion peptides that act in coordination has been suggested **[8]**; one of the peptides would promote the dehydration process while the other one would act in modifying/disturbing the lipid organization within the target membrane **[19,21,23]**. Hence, the catalytic role of the fusion peptide(s) is likely to tackle three properties of the target membrane in the virus entry machinery: (i) dehydration of the intermembrane space for the fusing membranes coming into the required proximity, (ii) to promote negative curvature to form the hemifusion stalk, and (iii) To alter the lipid packing density which will be generated in the highly curved local dimples of the stalk **[15,21]**. The effectiveness of these three processes is likely to depend upon the membrane lipid composition. Further research is devoted to this issue, and new clues are expected to come from electron and fluorescence microscopy **[24]**.

2.2. Raft lipids related to virus entry

Because of the dominant phospholipid in the outer leaflet of most membranes is the bilayer-forming, positive charged diacylglycerophosphatidylcholine (PC), the idea was early raised that the viral docking to the receptor on the target cell and, consequently, the membrane fusion were likely to take place at specific microdomains with particular lipid composition, the so-called lipid-rafts **[6,20,25–29]**. A special characteristic of the lipid-rafts is the high content of cholesterol (Chol) **[30–32]**. Even though high content of sphingolipids and gangliosides is also a defining characteristic of lipid rafts (Figure 1), direct *in vivo* visualization still remains unresolved **[32]**.

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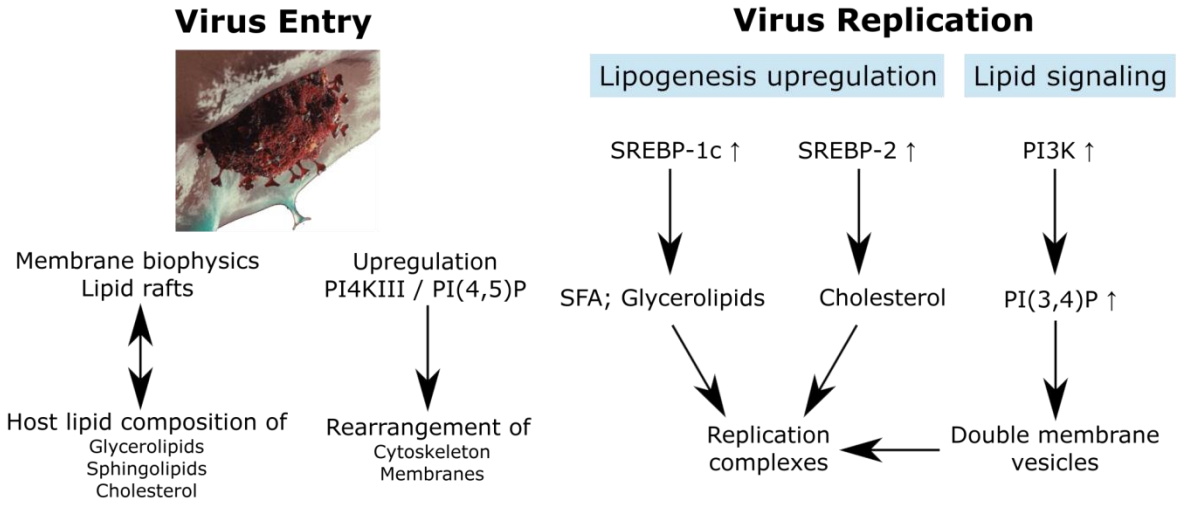

Figure 1. Relationship between the virus entry and replication with the lipidome. SREBP, sterol regulatory element binding protein; SFA, saturated fatty acid. SARS-CoV-2 artwork was modified from a work from *We Are Covert*, who allows anyone to use it for any purpose including unrestricted redistribution, commercial use, and modification.

An unexplored possibility is that rafts do not have a permanent localized existence, but they arise under the induction of certain proteins like the hydrophobic insert of the viral fusion peptide or the fusion loop. This fact might be also responsible for bringing negatively charged lipids from the inner leaflet of the bilayer to its outer leaflet by flip-flop mechanisms. This hypothesis would explain the promotion of virus entry by the interaction of the fusion peptide with the negatively charged phospholipid headgroups **[18,20]**, as well as the kinetics of the membrane fusion **[18]**. A number of studies have shown that increasing the relative concentration of Chol in the bilayer composition the hemifusion step and pore widening are speed-up, whereas either depletion of Chol in the cell culture medium or inhibition of Chol synthesis by statins was able to halt the viral infection at the virus entry step **[19–21,33,34]**. The effect of Chol promoting membrane merging has also been observed for Bis-(monoacylglycero)-phosphate (BMP) **[19]**. This particular phospholipid was shown to be strictly necessary for Dengue virus (DENV) entry even at low endosomal pH **[35]**. As pointed out above, the exact role played by Chol is not known in detail, but its intrinsic negative curvature seems to be an essential characteristic in promoting the stalk formation during viral entry. However, a recent study shows that the Chol action is likely to involve direct influence on the oligomeric state of the fusion peptide after insertion into the host cell membrane, as well as the effects of the fusion peptide on the membrane reorganization and dynamics **[36]**. In another recent study, a new lipid-label-free methodology was used to measure the kinetics of influenza virus infection **[37]**. According to the results of this study, Chol is able to augment the efficiency of membrane fusion in receptor-binding-independent manner. Nevertheless, the rate of membrane fusion was not altered. This leads the authors to conclude that the positive effect of Chol in membrane lipid mixing is related to its capability to induce negative curvature. Since membrane mixing was achieved in this latter study without binding of the spike protein of the influenza virus to the host cell receptor, the catalytic effect of the fusion peptide might run in an independent way in this virus. Cleavage of the spike protein in SARS-CoV-1 seems also not to be necessary for the fusion peptide to become fusogenic, but rearrangement of disulfide bridges in the S1 peptide after receptor binding are likely involved in the conformational changes driving the fusion mechanism **[36,38]**. Contrary to these latter results, which point to the fact that membrane fusion is independent of viral protein attachment to its receptor, Guo et al. reported lipid-raft dependent viral protein binding with suppression of viral infection if the lipid-rafts were disrupted with cholesterol drug-induced depletion; lipid-rafts, as recognized by the caveolin-1 marker, were the membrane domain that

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co-localized structural proteins of the infectious bronchitis virus (IBV) but were not the nonstructural proteins **[28]**. The question as to whether the lipid-raft domains may serve as platforms to concentrate the proteins required for viral entry and, even though some evidence exists, to activate signaling pathways inside the host cell still remains unsolved.

Sphingomyelins (SMs) are also typical lipids found in lipid-rafts, which contribute to make these membrane microdomains detergent resistant **[27]**. The structure of a representative of this lipid class is illustrated in Figure 2. The ganglioside GM1, a sphingolipid, is used as marker of lipid-rafts **[27]**. Sphingolipids (SLs) promote to an extent higher than Chol the liquid ordered phase in the outer leaflet of the membrane bilayer because of the long saturated acyl chains they currently contain (R group in Figure 2 may extend for up to 26 C), in addition to their capability to form intermolecular hydrogen bonds **[39]**. A relevant function of lipid-rafts has been suggested to be the connection between the events outside the cell with the pathways inside the cell, thus acting as 'signaling platforms'. With the aim of this function to be properly accomplished, lipid-rafts would act as concentrators of specific transmembrane proteins, mainly receptors, whose compatibility with the membrane phase would determine their selectivity. Thus, SLs would account for a role in connecting the outer leaflet with the inner leaflet through their long saturated acyl chains. Regarding virus entry, research has been primarily focused towards the role played by Chol, but a number of studies have also enlightened the SM influence on this early step of viral infection. Displacement of Chol by SMs and the other way round has been demonstrated, with the liquid-ordered bilayer phase being preferentially determined by the interaction between SM and Chol. This interaction would be controlled to a certain extent by the intracellular actin meshwork, which would also be responsible for the compartmentalization of the membrane into lipid specific domains **[40]**. Furthermore, the actin role is possibly extended to the routing of the viral genomic material towards the replication place inside the host cell. Hydrolysis of SM by sphingomyelinases to render the corresponding Ceramide (Cer) in specific membrane domains is proposed to regulate the dynamics of Chol in the cell membrane, with progressive disassembly of Chol from the liquid-ordered phase and its displacement. Because of the interaction of Cer with Chol has been suggested to be an apoptotic regulator, it can be expected that viral proteins would act in recruiting Chol to displace Cer and to avoid the programmed cell dead. This is added to the other characteristics conferred by Chol to the membrane mechanical properties as discussed above. To study the influence of ceramide Chol-containing PC plus PE liposomes on membraine fusion of Semliki Forest Virus (SFV, Alphavirus family, Togaviridae), ceramide analogs have been used **[41]**. According to this experiment, the role played by the 3-hydroxyl group and the 4,5-*trans* carbon-carbon double bond of the sphingosine backbone were found to be essential in fussion process (Figure 2). In additon, ceramide was the simplest SL to accomplish this significant contribution in mediating the fussion, independently of the length of the acyl chain. More recently, a Ca^{2+}-dependent pathway of infection by the Rubella virus (RuV, Rubivirus family, *Togaviridae*) was demonstrated to proceed through direct binding of the fusion loop in the viral E1 protein to SM/Chol enriched membranes **[42]**. However, treatment of host cells with sphingomyelinase proved that SM is exclusively required for viral entry but is not for the further steps of viral replication. SM in the host cell membrane and acid sphingomyelinase (ASMase) activity have also been shown to be required by the Ebola virus (EBOV), a negative single stranded RNA virus belonging to the *Filoviridae* family, to get into the host cell. The ASMase activity renders ceramide that provoques raft enlargement and membrane invagination **[43]**. This study also showed that the virus was able to recruit both SM and ASMase to the raft where the viral attachment was happening. Conversely, Bovine herpesvirus 1 (BoHV-1, *Herpesviridae* family) seems to require SM in the virus envelope but not in the host cell **[44]**. The role played by ceramides is rather contradictory as they may enhance or inhibit virus replication, but this SL seems to be related rather to the viral replication phase **[45–47]**. In virus using the endocytic pathway, as the Influenza virus or the Ebola virus, it has been shown that activity of glucosylceramidase (GBA) is required for viral entry and membrane fusion through regulation of endocytosis, but in a virus-dependent manner. It was also shown that trafficking of the epidermal growth factor (EGF) to late endosomes was impaired in GBA-knockout cells, a fact that affects

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negatively virus entry through spoiling the endocytic pathway **[48]**. Indeed, co-clustering of the HA attachment factor and EGF in submicrometer domains that overlap partially has been reported recently **[49]**. Accordingly, there is evidence that SLs have a function in enveloped ssRNA viruses at the early stage of infection through the viral entry modulation, but further research is still necessary to unveil the exact mechanisms of SLs reactions.

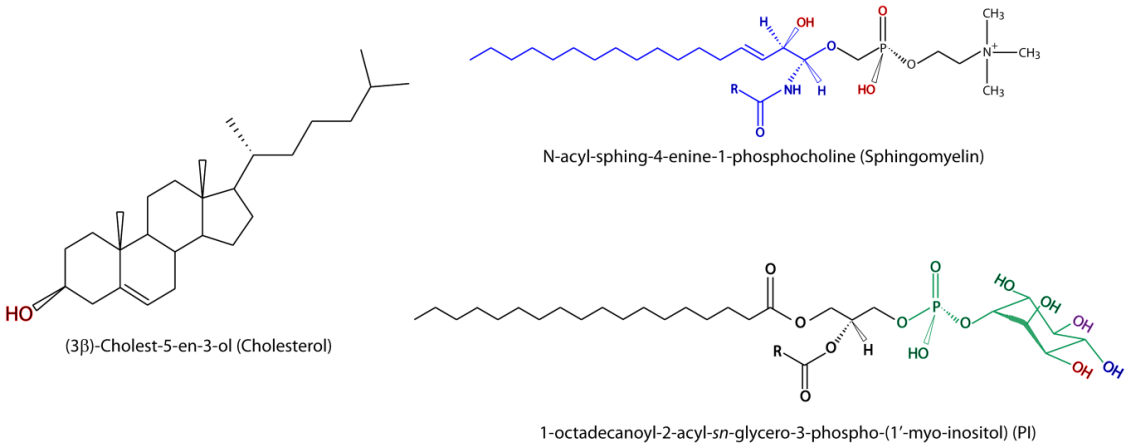

Figure 2. Schematic representation of the structure of the most relevant lipids in virus infection. Hydroxyl (HO) and oxigen (O) atoms potentially involved in the interaction with the fusion peptide or fusion loop are remarked in red in cholesterol and sphingomyelin. The basic ceramide structure is marked in blue in the sphigomyelin structure. In phosphatidylinositol (PI), the hydroxyl groups that can be esterified with phosphate at the positions 3, 4 and 5 of the myo-inositol group to render PIP (PI3P or PI4P), PIP2 (PI(3,4)P or PI(4,5)P) and PIP3 (PI(3,4,5)P) are marked in red, blue and violet, respectively.

Some CoVs (HCoV-OC43 and HCoVHKU1), as well as influenza A virus (hemagglutinin, HA) and other non-related viruses (i.e. non-enveloped simian virus 40 SV-40, of polyomavirus family), use the sialoglycan moiety (9-*O*-acetyl-sialic acid) of gangliosides or glycoproteins located in membrane lipid-rafts as receptors for the spike protein. The amino acid Trp90 in the domain A of the HCoV-OC43 S protein was shown to be essential for receptor binding. However, despite the fact that binding to 9-*O*-acetyl-sialic acid is required for membrane fusion, further interaction of the virus protein with other host membrane sialoglycans or proteins is necessary to induce the conformational changes leading to membrane fusion **[50,51]**. Conversely, formation of the complex SV40 protein with the host cell ganglioside GM1 was found to be enough to induce membrane curvature and invaginations necessary for membrane fusion **[52]**.

As already discussed above, some studies have depicted the possibility that interaction of the fusion peptide or fusion loop with negatively charged phospholipids on the host membrane might be required for efficient membrane fusion **[18]**. In this regard, phosphatidylserine (PS) in the virus envelope has been demonstrated to serve after externalization as virus co-receptor through the T cell immunoglobulin mucin domain 1 (TIM-1) receptor in EBOV and other viruses, even in an indispensable fashion **[53–56]**. In the study of Nanbo *et al.* **[56]**, flipping of PS from the inner leaflet to the outer leaflet of the cell membrane for virion adquisition and incorporation to its envelope is proposed as a previous step to TIM1 binding. In herpes simplex virus (HSV), phospholipid scramblase-1 (PLSCR1), after activation by HSV exposure, flips both PS and Akt to the outside of the membrane in a Ca^{2+}-dependent mechanism. PS is restored to the inner leaflet 2 to 4 h after infection to avoid apoptotic triggering **[55]**, suggesting a different role for PS in relation to the TIM-1 PS receptor. However, the function of TIM-1 as essential receptor for HAV has been disputed

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[57] due to quasi-enveloped HA virions (eHAV) were able to infect TIM1-knockout Vero cells to a similar extent to naked HAV. Hence, the authors proposed TIM1 to be an accessory attachment factor by binding PS on the HAV envelope rather than an essential virus protein receptor. In spite of these contradictory data, PS seems to act in any way in virus attachment and entry in certain virus families, at least contributing to an effiecient process, but the exact role may depend on every virus or it may be complementary to other factors.

A phospholipid currently associated to the inner leaflet in lipid-rafts is phosphatidylinositol (PI), it is a negatively charged phospholipid with important and versatil signaling functions (Figure 2) **[58,59]**. Abundant data suggest that a derivative of PI, the phosphatidylinositol 4,5-biphosphate (PIP2), accumulates preferently in liquid-disordered phases (L_d) **[8]**, where the Chol content is presumed to be low, interplaying with PS, which is rather localized in liquid-ordered phases (L_o).

PIs play an essential role also in endosome maturation, which is a requisite for virus infection of those using the endosomal pathway **[49,59]**. HIV infection, PIP2 has been proposed to coordinate the actin cytoskeleton changes required for efficient virus entry in CD4+ T cells **[60]**; after virus attachment to the host cell receptor, PIP2 is recruited to the binding membrane microdomain, and, in this way, PIP2 controls the proteins' reactions leading to actin polymerization. As well in HIV-1, the requisite of PIP2 accumulation for the virus Gag protein to be properly anchored and stabilized in the inner leaflet of the cell plasma membrane has been pointed out **[61,62]**. Two isoforms, α and γ, of the phosphatidylinositol-4-phosphate 5-kinase family type 1 (PIP5K1) have recently been shown to participate in Gag stabilization by PIP2 through targeting the Gag precursor $Pr55^{Gag}$ to the cell plasma membrane **[63]**. As commented above, interaction with the headgroup of negatively charged phospholipids like PS or PI may also contribute to the dehydration process in the formation of the hemifusion stalk by promotion of the inverted hexagonal phase in the lipid bilayer and binding of Ca^{2+} **[18]**. In *in vitro* experiments with COS-7 cells and multilamellar vesicles (MLVs), unspecific binding of the Marburg virus (MARV) mVP40 protein to PIP, PIP2 and even PIP3 species in MLVs, both in the presence or absence of PS, has been reported. In this study, it was also found that with increasing PS concentration the association of mVP40 to MLVs rose up to a threshold. Furthermore, adding sphingosine to reduce the negative charge load in the inner leaftet of the COS-7 cells decreased the binding level. These facts suggest that the electronic density, rather than the specific lipid species, is a determinant factor for binding **[64]**. Activation of the PI3K pathway for signaling is one of the most relevant features taking place for both entry and budding during infection by a number of viruses **[51,65–67]**. PI3K converts PIP2 into phosphatidylinositol 3,4,5-triphosphate (PIP3). In addition to stabilizing proteins or serving as binding factor, PIP2 has been shown to collaborate with Akt through the signaling pathway PI3K/Akt on avoiding apoptotic events and thus keeping the host cell metabolically active for virus replication and budding **[65–67]**.

All these results clearly bring to evidence that the lipid environment surrounding proteins involved in virus infection has a relevant function in the virus entry mechanism. Different lipids are essential for virus docking to the cell receptor either serving directly as (co)-receptors or providing the appropriate environment (lipid-rafts) for the necessary reactions (*e.g.* membrane curvature). In addition, virus, through specific protein conformational changes, takes advantage of several cell signaling pathways controlled by diverse membrane lipids. This process allows virus to govern the cell metabolism following endocytosis of the viral genetic molecules.

3. Lipid regulation in virus replication: viral factories.

After the virus or its genome gets inside the infected cell, ssRNA+ viruses and other enveloped ones that replicate in the cytoplasm manage the cell metabolism to develop the replication scaffold, this membrane structure bolstering the so called 'virus factory' **[5,51,68–74]**. There is consensus on that functions of these structures are (i) to compartmentalise the diverse processes involved in viral genome replication, its envelopment and structural protein assembly; (ii) to increase virion concentration during budding before infecting naïve cells; and (iii) to create a protected

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environment to scape of innate immune recognition of the viral components. Virus replication imposes an extra-energetic expenditure to the cell metabolism. Hence, cell central metabolism is orchestated by viral proteins to redirect towards the generation of energy enough and metabolites that are required for virus replication. In particular, building the scaffold demands a high rate of new lipid synthesis. Therefore, the lipid metabolism is hijacked by the virus proteins for the *de novo* synthesis of fatty acids in order to generate the scaffold membranes, the replication complexes (RCs), as well as for energy production in the β-oxidation pathway in the mitochondria. Concurrently, the cell metabolism needs to be kept above a threshold level to avoid exhaustion of the host cell. Full understanding of the mechanisms and related factors involved in virus-host interaction is a requisite for developing efficient antiviral infection therapies.

3.1. Viral replication complexes.

The scaffold structure raised for building the viral factory varies between different virus in their morphology and, possibly, lipid composition. Flaviviruses develop a so called 'membranous network' (MN) in a spherule/invagination type, while does coronavirus through quarters-like delimited by 'double membrane vesicles' (DMVs type). Nonetheless, HCV (*Flaviviridae*) uses DMVs instead **[71]** and, hence, this morphological separation may have exceptions or be somewhat diffuse. An extended review of the different virus family-related morphologies of the MNs as well as diverse factors influencing their formation can be found in **[69]**. It should be remarked that the exact lipid composition of the RCs' membranes is not known in detail yet, although there is evidence that their lipid profile differs from that of the organelles from which they are generated. Enrichment of typical lipids like Chol, SMs and glycosphingolipids in lipid-rafts seems to be a common feature of these MNs. The RCs' membranes may be originated from the endoplasmic reticulum (ER) in the perinuclear area, as for example in SARS-CoV and *Faviviridae* **[69,72,74]**, from the Golgi, giving rise to cytopathic vesicles (CPVs) as in *Togaviridae* and *Picornaviridae* **[69]**, from mitochondria (*Nodaviridae*) **[73]**, or from the cell plasma membrane (CPVs in Alphaviruses) **[69]**. However, vesicle trafficking between the ER and the Golgi organelles may contribute to an undefinition in this regard. MNs, and in particular DMVs, are connected to the cytosol through a pore, which is believed to serve as the gate to the replication scaffold for the requiered metabolites, in particular nucleotides. This pore-mediated gate has not been detected up to date in SARS-CoV's DMVs, a fact that raises the concern on how the required metabolites get inside the RCs. There is evidence from a number of studies that DMVs are the site of replication, but it has also been shown that DMVs can be developed irrespective of whether RNA replication takes place by the sole action of the viral proteins, at least for HCV **[75,76]**. Viral non-structural proteins nsp3, nsp4 and nsp6 are involved in DMV development in SARS-CoV-1 in a time-dependent manner, and correlating with RNA replication. Timecourse events have been shown to run with initial formation of single membrane vesicles (SMVs) during the first 2-4 h of cell infection, which futher evolve to DMVs 16 h after infection, and they ultimately turn into multimembraneous vesicles (MMVs) close to the *cis*-Golgi at the budding stage 36-48 h after infection, this latter transformation being coincident with the formation of vesicle packets **[69,72,73,77]**. In HCV, NS5A seems to be enough for DMV formation, but collaboration of NS3-5B is required for completing efficient DMVs, whereas NS4B is likely the responsible of inducing formation of SMVs **[71,74,76]**. Even though particular hints can be likely associated to every particular virus, there are common features shared by all ssRNA+ viruses regarding RCs' structure and buildup.

3.2. Lipid-related host factors associated to the RCs' buildup

Enveloped viruses like ssRNA+ viruses have a membrane lipid whose profile is different respect to the original organelle membrane when the envelope is created. Since viral membrane is known to be enriched in Chol, SLs and PPLs with saturated acyl chains, the DMV is believed to be also primarily composed of such type of lipids. An unusual SL, dehydrosphingomyelin, along with plasmalogens of PE, and PS were reported in HIV envelope **[78]**. A role for

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sphingomyelin-to-ceramide conversion has been proposed in WNV budding as its envelope was found to be highly enriched in SM **[79]**. More recently, using multi-color super-resolution microscopy and mass spectrometry analysis, a substantial increase in PIP2 (from 11% to 51%), and PIP3 (from 0.01% to 0.13%), was reported in HIV membrane as compared with the plasma membrane of the host cell **[62]**; this fact is related to the recruitment of Gag protein for efficient membrane fusion as aforementioned (Figure 1).

However, the most striking and known lipid-related factor associated to the MNs' development is the PI4KIII signaling pathway. PI4Pα isoform, which is mainly expressed in the ER, has been shown as a key factor for HCV replication, whereas the PI4KIIIβ is found in the Golgi as required by Picornaviruses and some HCV strains **[69]**. This enzyme interacts with the viral protein NS5A, and disrupting this interaction prevents virus replication. The product of the PI4K enzyme is PIP4; enrichment in this PI has been shown to act in different processes regarding virus replication: membrane curvature, directly or indirectly through recluting Chol **[80]**, glycosphingolipid transport to the RCs through the FAPP2 protein **[81]**, and protein concentration. However, conversely to these studies, it has been shown that currently used inhibitors of PI4KIIIα, enviroxime and BF738735, actually exert its inhibition against PI3K **[82]**. Thus, this result points out a genomic dependence on the PI kinases in HCV; or otherwise, the action on PI3K is rquired only at the entry stage (see above). Enviroxime-like inhibitors have been shown to halt enterovirus replication through the action against PI4Kβ **[83]**. The *de novo* lipid synthesis has also been evidenced for WNV, from the *Flaviviridae* family as HCV, to proceed in a PI4P-independent fashion and, concurrently, not related to PI4KIII signaling **[84]**. There is no clear evidence of the PI4K signaling pathway has a relevant function in MNs' development. Hence, while PI4KIIIβ was shown to be important for SARS-CoV's DMV formation **[85]**, another study did not find its metabolite, PI4P, within the host factors involved in SARS-CoV replication, and the authors attibute to PI4P a function rather in virus entry. However, the authors of this latter study acknowledge that siRNA methodology may provide false negatives **[86,87]**. Because DMVs are not common in healthy cells but they can be observed during authophagy, it has been suggested that SARS-CoV and other coronaviruses use the autophagy pathway for development of the DMVs; indeed it has been shown that nsp6 in MHV or the equivalent nsp5-7 in arteriviruses, which hit the ER, can activate such pathway **[73,88]**. Nonetheless, DMVs are smaller than autophagosomes and, hence, they might be rather EDEsomes enriched in PI3P and not follow exactly the same synthetic route [88]. The autophatocytic pathway has also been associated to the start of HCV infection but it seems not to be necessary for the infection to go on **[76]**.

Similar to viral entry, Chol has been found to be also relevant in the RCs' membranes **[73,76]**. Up to c.a. 9-fold enrichment of Chol was found in HCV-developed DMVs as compared to its content in the ER membranes from which DMVs were originated [71]. A key protein in Chol metabolism associated to non-vesicular transport is the oxysterol binding protein (OSBP). This protein has been described to transport Chol to PI4P-enriched membranes, which would agree with its collaboration in delivering Chol to DMVs with abundant content of this PI **[71]**. The ceramide transfer protein (CERT) and the four-phosphate adaptor protein 2 (FAPP2) are known to undergo a similar fate in HCV infection **[76]**. An important protein involved in cellular lipid homeostasis is the sterol regulatory element binding protein (SREBP), a bHLH-zip transcription factor with three isoforms; SREBP1c regulate expression of fatty acid (FA) biosynthesis genes, whereas SREBP2 transactivate genes implied in Chol biosynthesis, intracellular lipid transport and lipoprotein import **[89]**. A recent study shows that inhibition of SREBP with the retinoid derivative and RAR-α agonist AM580 prevents MERS-CoV infection by avoiding formation of functional DMVs **[90]**. In this study, the lipid metabolism was the most affected pathway, with sterol biosynthesis being strengthened at expenses of the glycerophospholipid metabolic pathways. Fast activation of the lipid biosynthesis enzymes Acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and HMG-CoA synthase (HMGCS) was observed in such study, whose activity was partially blocked by AM580 inhibition of SREBP enzymes. Promotion of lipid biosyntheis after infection had already been pointed out for

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HCV in an elegant proteomics and lipidomics study **[91]**. HCV infection elicited changes in the proteome of host cells that resembled the Warburg effect described in cancer cells towards lactate production and support of continuous glycolysis; concurrently, up-regulation of citrate synthase (CS) and other lipogenic enzymes 24h after infection was interpreted by the authors of the latter study as indicative of re-routing of the TCA cycle for cytosolic accumulation of citrate, which would be used in FA synthesis. Up-regulation of peroxisomal and mitochondrial FA oxidation pathways paralleled the other metabolic changes. An increase in pro-apoptotic ceramides was observed in the latter study as well; two possible interpretations were attributed to this finding, either a cytopathic effect after cell cycle arrest over time enough to complete virus offspring, or a defense response of the host cell to avoid infection spread.

4. Additional pathways of lipid metabolism affected in virus infection.

Remodelling of the lipid metabolism by virus infection may leave signals at the organism level even some years after healing. The metabolome profile of patients undergoing SARS-CoV-1 infection during the outbreak of 2002-2003 was assessed 12 years after overcoming the pathology **[92]**. An outstanding result of this study regarding disturbed lipid metabolism was the elevation of phosphatidylinositols (PIs) and lysophosphatidylinositols (LPIs) concentrations in serum, which in turn correlated positively with the levels of very low-density lipoproteins (VLDL); higher concentrations of products of the phospholipase A_2 (PLA_2) like lysophospholipids (LPPLs) and free arachidonic acid (AA) were also also found in patients as compared to healthy voluntiers, the level of AA correlating with the ratio of LPI(18:0) to total 18:0-PIs. These results show a potential high sensitivity of SARS-CoV patients to PLA_2 activity. In the general context, the patients' metabolome pointed to hyperlipidemia, cardiovascular abnormalities and glucose metabolism alteration; even though the authors acknowledge that some metabolic disturbations are likely owed to the pharmacological treatment. High levels of PLA_2 group IID (PLA2G2D) in lungs of middle-aged mouse as compared to young mouse had previously been associated to a fatal or worse outcome **[93]**. The authors of this study conclude that the negative influence of this enzyme in SARS-CoV infection was to increase the concentration of anti-inflammatory lipid mediators, mainly protaglandin D_2 (PGD_2), which impaired the efficient function of the immune system **[94]**. In the recent SARS-CoV-2 outbreak (COVID-19), mortality has mostly affected aged people above 60 years old, thus showing an age-related fatality as for SARS-CoV-1 and MERS-CoV **[95]**. Using a lipidomics approach, the effect of HCoV-229E and MERS-CoV infection on the host cell lipid profile was recently investigated in cell culture **[96]**. Main conclusions of this study agree with the rised content of AA and LPPLs through PLase activity, which points out that possible virus-induced activation of $cPLA_2$ favors virus replication as a factor required for DMVs' formation. In this study, linoleic acid (LA) or AA supplementation to the culture cells suppresed replication, demonstrating the perturbation of the LA/AA axis on the lipid metabolism.

In the COVID-19 outbreak it has been suggested that increasing the levels of vitamin D could help fighting against the SARS-CoV infection **[97]**. This suggestion is based on the fact that 25-hydroxyvitamin D3 was found to protect Huh7 cells against MERS-CoV **[90]**. Vitamin D is a lipid related compound belonging to the group of fat-soluble secosteroids, with the most important form in humans being vitamin D3 (cholecalciferol) **[98]**. In a recent study, high doses of vitamin D have shown protective effect against DENV infection through regulating Toll-like receptor expression and modulation of pro-inflammatory cytokines release, suggesting that its action is focused towards the immune system modulation rather than to lipid metabolism **[99]**. However, evidence on the beneficial effects of vitamin D uptake is still poor and more studies are devoted to this issue.

Lipids, as components of membranes, are related to viroporins, specific viral proteins that are known to create ion channels for ion trafficking **[100–102]**. The effect on cell metabolism of diverse viroporins differs among them but there is evidence that they are closely related to viral pathogenity **[100]**. Viroporins may play a relevant role during virus infection as they are involved in membrane

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permeability and calcium homeostasis. Their participation in the develpment of vacuoles from the ER during the DMVs' formation has been suggested, but data on this issue still scarce. Regulating Ca^{2+} viroporins could favor the membrane fusion through the interaction of this cation with the phospholipid headgroups and, concurrently, facilitating the required dehydratation reaction. Viroporins are not required for virus replication with the exception of rotavirus and picornavirus; concerning whether this function is exerted through the ion channels or another property of viroporins remains still unknown **[100]**. The lipid composition of the membrane may influence the viroporin activity, leading to different versions of ion channels depending on the electric charge that the phospholipids confer to the membrane and curvature **[102]**. Further research is necessary to understand the role played by viroporins in virus infection in order to consider them as potential therapeutic targets.

5. Conclusions

Remodelling of the virus-induced host cell lipid metabolism is a standing-out feature of the viral infection. Main actors are well known to be Chol, SLs, and PIs, but other lipid species and their related pathways like the LA/AA axis are also relevant. How to target the lipid metabolism in a safe manner to avoid virus infection or reduce its pathogenity is a promising therapeutic tool, but it demands improving the knowledge on the actual pathways that are affected over the virus life cycle. The exact mechanism through which the enzyme inhibitors act on the key enzymes of lipid metabolism is also required to develop more efficient and safe therapeutic drugs. Because lipid metabolism is essential for proper cell function, selective drugs targeting the virus or exclusively the infected cells have to be used to avoid dangerous side-effects.

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References

1. Wu, F.; Zhao, S.; Yu, B.; Chen, Y.-M.; Wang, W.; Song, Z.-G.; Hu, Y.; Tao, Z.-W.; Tian, J.-H.; Pei, Y.-Y.; et al. A new coronavirus associated with human respiratory disease in China. *Nature* **2020**, *579*, 265–269, doi:10.1038/s41586-020-2008-3.
2. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19. Available online: https://www.who.int/emergencies/diseases/novel-coronavirus-2019, (accessed on 09 June 2020).
3. Fehr, A.R.; Perlman, S. Coronaviruses: An Overview of Their Replication and Pathogenesis. In *Coronaviruses*; Maier, H.J., Bickerton, E., Britton, P., Eds.; Methods in Molecular Biology; Springer New York: New York, NY, 2015; Vol. 1282, pp. 1–23 ISBN 978-1-4939-2437-0.
4. Reid, C.; Airo, A.; Hobman, T. The Virus-Host Interplay: Biogenesis of +RNA Replication Complexes. *Viruses* **2015**, *7*, 4385–4413, doi:10.3390/v7082825.
5. Novoa, R.R.; Calderita, G.; Arranz, R.; Fontana, J.; Granzow, H.; Risco, C. Virus factories: associations of cell organelles for viral replication and morphogenesis. *Biology of the Cell* **2005**, *97*, 147–172, doi:10.1042/BC20040058.
6. Baltimore, D. Expression of animal virus genomes. *Bacteriol Rev* **1971**, *35*, 235–241.
7. Marsh, M.; Helenius, A. Virus Entry: Open Sesame. *Cell* **2006**, *124*, 729–740, doi:10.1016/j.cell.2006.02.007.

Peer-reviewed version available at Metabolites 2020, 10, 356; doi:10.3390/metabo10090356

8. Yang, S.-T.; Kreutzberger, A.J.B.; Lee, J.; Kiessling, V.; Tamm, L.K. The role of cholesterol in membrane fusion. *Chemistry and Physics of Lipids* **2016**, *199*, 136–143, doi:10.1016/j.chemphyslip.2016.05.003.
9. Ou, X.; Zheng, W.; Shan, Y.; Mu, Z.; Dominguez, S.R.; Holmes, K.V.; Qian, Z. Identification of the Fusion Peptide-Containing Region in Betacoronavirus Spike Glycoproteins. *J. Virol.* **2016**, *90*, 5586–5600, doi:10.1128/JVI.00015-16.
10. Zhang, W.; Zheng, Q.; Yan, M.; Chen, X.; Yang, H.; Zhou, W.; Rao, Z. Structural characterization of the HCoV-229E fusion core. *Biochemical and Biophysical Research Communications* **2018**, *497*, 705–712, doi:10.1016/j.bbrc.2018.02.136.
11. Zeisel, M.B.; Felmlee, D.J.; Baumert, T.F. Hepatitis C Virus Entry. In *Hepatitis C Virus: From Molecular Virology to Antiviral Therapy*; Bartenschlager, R., Ed.; Current Topics in Microbiology and Immunology; Springer Berlin Heidelberg: Berlin, Heidelberg, 2013; Vol. 369, pp. 87–112 ISBN 978-3-642-27339-1.
12. Zhu, Y.-Z. How hepatitis C virus invades hepatocytes: The mystery of viral entry. *WJG* **2014**, *20*, 3457, doi:10.3748/wjg.v20.i13.3457.
13. Chigbu, D.; Loonawat, R.; Sehgal, M.; Patel, D.; Jain, P. Hepatitis C Virus Infection: Host–Virus Interaction and Mechanisms of Viral Persistence. *Cells* **2019**, *8*, 376, doi:10.3390/cells8040376.
14. Plemper, R.K. Cell entry of enveloped viruses. *Current Opinion in Virology* **2011**, *1*, 92–100, doi:10.1016/j.coviro.2011.06.002.
15. Harrison, S.C. Viral membrane fusion. *Virology* **2015**, *479–480*, 498–507, doi:10.1016/j.virol.2015.03.043.
16. Benhaim, M.A.; Lee, K.K. New Biophysical Approaches Reveal the Dynamics and Mechanics of Type I Viral Fusion Machinery and Their Interplay with Membranes. *Viruses* **2020**, *12*, 413, doi:10.3390/v12040413.
17. Ivanovic, T.; Choi, J.L.; Whelan, S.P.; van Oijen, A.M.; Harrison, S.C. Influenza-virus membrane fusion by cooperative fold-back of stochastically induced hemagglutinin intermediates. *eLife* **2013**, *2*, e00333, doi:10.7554/eLife.00333.
18. Chao, L.H.; Klein, D.E.; Schmidt, A.G.; Peña, J.M.; Harrison, S.C. Sequential conformational rearrangements in flavivirus membrane fusion. *eLife* **2014**, *3*, e04389, doi:10.7554/eLife.04389.
19. Aeffner, S.; Reusch, T.; Weinhausen, B.; Salditt, T. Energetics of stalk intermediates in membrane fusion are controlled by lipid composition. *Proceedings of the National Academy of Sciences* **2012**, *109*, E1609–E1618, doi:10.1073/pnas.1119442109.
20. Gui, L.; Ebner, J.L.; Mileant, A.; Williams, J.A.; Lee, K.K. Visualization and Sequencing of Membrane Remodeling Leading to Influenza Virus Fusion. *J. Virol.* **2016**, *90*, 6948–6962, doi:10.1128/JVI.00240-16.
21. Ge, M.; Freed, J.H. Two Conserved Residues Are Important for Inducing Highly Ordered Membrane Domains by the Transmembrane Domain of Influenza Hemagglutinin. *Biophysical Journal* **2011**, *100*, 90–97, doi:10.1016/j.bpj.2010.11.014.
22. Basso, L.G.M.; Vicente, E.F.; Crusca, E.; Cilli, E.M.; Costa-Filho, A.J. SARS-CoV fusion peptides induce membrane surface ordering and curvature. *Sci Rep* **2016**, *6*, 37131, doi:10.1038/srep37131.
23. Kuhl, T.; Guo, Y.; Alderfer, J.L.; Berman, A.D.; Leckband, D.; Israelachvili, J.; Hui, S.W. Direct Measurement of Polyethylene Glycol Induced Depletion Attraction between Lipid Bilayers. *Langmuir* **1996**, *12*, 3003–3014, doi:10.1021/la950802l.
24. Mahajan, M.; Bhattacharjya, S. NMR structures and localization of the potential fusion peptides and the pre-transmembrane region of SARS-CoV: Implications in membrane fusion. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2015**, *1848*, 721–730, doi:10.1016/j.bbamem.2014.11.025.
25. Carravilla, P.; Nieva, J.L.; Eggeling, C. Fluorescence Microscopy of the HIV-1 Envelope. *Viruses* **2020**, *12*, 348, doi:10.3390/v12030348.

Peer-reviewed version available at Metabolites 2020, 10, 356; doi:10.3390/metabo10090356

26. Lu, Y.; Liu, D.X.; Tam, J.P. Lipid rafts are involved in SARS-CoV entry into Vero E6 cells. *Biochemical and Biophysical Research Communications* **2008**, *369*, 344–349, doi:10.1016/j.bbrc.2008.02.023.
27. Earnest, J.T.; Hantak, M.P.; Park, J.-E.; Gallagher, T. Coronavirus and Influenza Virus Proteolytic Priming Takes Place in Tetraspanin-Enriched Membrane Microdomains. *J. Virol.* **2015**, *89*, 6093–6104, doi:10.1128/JVI.00543-15.
28. Guo, H.; Huang, M.; Yuan, Q.; Wei, Y.; Gao, Y.; Mao, L.; Gu, L.; Tan, Y.W.; Zhong, Y.; Liu, D.; et al. The Important Role of Lipid Raft-Mediated Attachment in the Infection of Cultured Cells by Coronavirus Infectious Bronchitis Virus Beaudette Strain. *PLoS ONE* **2017**, *12*, e0170123, doi:10.1371/journal.pone.0170123.
29. Zhang, J.; Pekosz, A.; Lamb, R.A. Influenza Virus Assembly and Lipid Raft Microdomains: a Role for the Cytoplasmic Tails of the Spike Glycoproteins. *J. Virol.* **2000**, *74*, 4634–4644, doi:10.1128/JVI.74.10.4634-4644.2000.
30. Takeda, M.; Leser, G.P.; Russell, C.J.; Lamb, R.A. Influenza virus hemagglutinin concentrates in lipid raft microdomains for efficient viral fusion. *Proceedings of the National Academy of Sciences* **2003**, *100*, 14610–14617, doi:10.1073/pnas.2235620100.
31. van Meer, G.; Voelker, D.R.; Feigenson, G.W. Membrane lipids: where they are and how they behave. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 112–124, doi:10.1038/nrm2330.
32. Sezgin, E.; Levental, I.; Mayor, S.; Eggeling, C. The mystery of membrane organization: composition, regulation and roles of lipid rafts. *Nat Rev Mol Cell Biol* **2017**, *18*, 361–374, doi:10.1038/nrm.2017.16.
33. Levental, I.; Levental, K.R.; Heberle, F.A. Lipid Rafts: Controversies Resolved, Mysteries Remain. *Trends in Cell Biology* **2020**, *30*, 341–353, doi:10.1016/j.tcb.2020.01.009.
34. Biswas, S.; Yin, S.-R.; Blank, P.S.; Zimmerberg, J. Cholesterol Promotes Hemifusion and Pore Widening in Membrane Fusion Induced by Influenza Hemagglutinin. *Journal of General Physiology* **2008**, *131*, 503–513, doi:10.1085/jgp.200709932.
35. Chlanda, P.; Mekhedov, E.; Waters, H.; Schwartz, C.L.; Fischer, E.R.; Ryham, R.J.; Cohen, F.S.; Blank, P.S.; Zimmerberg, J. The hemifusion structure induced by influenza virus haemagglutinin is determined by physical properties of the target membranes. *Nat Microbiol* **2016**, *1*, 16050, doi:10.1038/nmicrobiol.2016.50.
36. Zaitseva, E.; Yang, S.-T.; Melikov, K.; Pourmal, S.; Chernomordik, L.V. Dengue Virus Ensures Its Fusion in Late Endosomes Using Compartment-Specific Lipids. *PLoS Pathog* **2010**, *6*, e1001131, doi:10.1371/journal.ppat.1001131.
37. Meher, G.; Bhattacharjya, S.; Chakraborty, H. Membrane Cholesterol Modulates Oligomeric Status and Peptide-Membrane Interaction of Severe Acute Respiratory Syndrome Coronavirus Fusion Peptide. *J. Phys. Chem. B* **2019**, *123*, 10654–10662, doi:10.1021/acs.jpcb.9b08455.
38. Liu, K.N.; Boxer, S.G. Target Membrane Cholesterol Modulates Single Influenza Virus Membrane Fusion Efficiency but Not Rate. *Biophysical Journal* **2020**, *118*, 2426–2433, doi:10.1016/j.bpj.2020.03.021.
39. Masters, P.S. The Molecular Biology of Coronaviruses. In *Advances in Virus Research*; Elsevier, 2006; Vol. 66, pp. 193–292 ISBN 978-0-12-039869-0.
40. Munro, S. Lipid Rafts. *Cell* **2003**, *115*, 377–388, doi:10.1016/S0092-8674(03)00882-1.
41. García-Arribas, A.B.; Alonso, A.; Goñi, F.M. Cholesterol interactions with ceramide and sphingomyelin. *Chemistry and Physics of Lipids* **2016**, *199*, 26–34, doi:10.1016/j.chemphyslip.2016.04.002.
42. Corver, J.; Moesby, L.; Erukulla, R.K.; Reddy, K.C.; Bittman, R.; Wilschut, J. Sphingolipid-dependent fusion of Semliki Forest virus with cholesterol-containing liposomes requires both the 3-hydroxyl group and the double bond of the sphingolipid backbone. *J. Virol.* **1995**, *69*, 3220–3223.

Peer-reviewed version available at Metabolites 2020, 10, 356; doi:10.3390/metabo10090356

43. Otsuki, N.; Sakata, M.; Saito, K.; Okamoto, K.; Mori, Y.; Hanada, K.; Takeda, M. Both Sphingomyelin and Cholesterol in the Host Cell Membrane Are Essential for Rubella Virus Entry. *J Virol* **2017**, *92*, e01130-17, doi:10.1128/JVI.01130-17.
44. Miller, M.E.; Adhikary, S.; Kolokoltsov, A.A.; Davey, R.A. Ebolavirus Requires Acid Sphingomyelinase Activity and Plasma Membrane Sphingomyelin for Infection. *Journal of Virology* **2012**, *86*, 7473–7483, doi:10.1128/JVI.00136-12.
45. Pastenkos, G.; Miller, J.L.; Pritchard, S.M.; Nicola, A.V. Role of Sphingomyelin in Alphaherpesvirus Entry. *J Virol* **2018**, *93*, e01547-18, /jvi/93/5/JVI.01547-18.atom, doi:10.1128/JVI.01547-18.
46. Soudani, N.; Hage-Sleiman, R.; Karam, W.; Dbaibo, G.; Zaraket, H. Ceramide Suppresses Influenza A Virus Replication *In Vitro*. *J Virol* **2019**, *93*, e00053-19, /jvi/93/7/JVI.00053-19.atom, doi:10.1128/JVI.00053-19.
47. Grassmé, H.; Riehle, A.; Wilker, B.; Gulbins, E. Rhinoviruses Infect Human Epithelial Cells via Ceramide-enriched Membrane Platforms. *J. Biol. Chem.* **2005**, *280*, 26256–26262, doi:10.1074/jbc.M500835200.
48. Aktepe, T.E.; Pham, H.; Mackenzie, J.M. Differential utilisation of ceramide during replication of the flaviviruses West Nile and dengue virus. *Virology* **2015**, *484*, 241–250, doi:10.1016/j.virol.2015.06.015.
49. Drews, K.; Calgi, M.P.; Harrison, W.C.; Drews, C.M.; Costa-Pinheiro, P.; Shaw, J.J.P.; Jobe, K.A.; Nelson, E.A.; Han, J.D.; Fox, T.; et al. Glucosylceramidase Maintains Influenza Virus Infection by Regulating Endocytosis. *J Virol* **2019**, *93*, e00017-19, /jvi/93/12/JVI.00017-19.atom, doi:10.1128/JVI.00017-19.
50. Sieben, C.; Sezgin, E.; Eggeling, C.; Manley, S. Influenza A viruses use multivalent sialic acid clusters for cell binding and receptor activation. *PLoS Pathog* **2020**, *16*, e1008656, doi:10.1371/journal.ppat.1008656.
51. Tortorici, M.A.; Walls, A.C.; Lang, Y.; Wang, C.; Li, Z.; Koerhuis, D.; Boons, G.-J.; Bosch, B.-J.; Rey, F.A.; de Groot, R.J.; et al. Structural basis for human coronavirus attachment to sialic acid receptors. *Nat Struct Mol Biol* **2019**, *26*, 481–489, doi:10.1038/s41594-019-0233-y.
52. Mazzon, M.; Mercer, J. Lipid interactions during virus entry and infection: Lipids and Viruses. *Cell Microbiol* **2014**, *16*, 1493–1502, doi:10.1111/cmi.12340.
53. Ewers, H.; Römer, W.; Smith, A.E.; Bacia, K.; Dmitrieff, S.; Chai, W.; Mancini, R.; Kartenbeck, J.; Chambon, V.; Berland, L.; et al. GM1 structure determines SV40-induced membrane invagination and infection. *Nat Cell Biol* **2010**, *12*, 11–18, doi:10.1038/ncb1999.
54. Moller-Tank, S.; Kondratowicz, A.S.; Davey, R.A.; Rennert, P.D.; Maury, W. Role of the Phosphatidylserine Receptor TIM-1 in Enveloped-Virus Entry. *Journal of Virology* **2013**, *87*, 8327–8341, doi:10.1128/JVI.01025-13.
55. Jemielity, S.; Wang, J.J.; Chan, Y.K.; Ahmed, A.A.; Li, W.; Monahan, S.; Bu, X.; Farzan, M.; Freeman, G.J.; Umetsu, D.T.; et al. TIM-family Proteins Promote Infection of Multiple Enveloped Viruses through Virion-associated Phosphatidylserine. *PLoS Pathog* **2013**, *9*, e1003232, doi:10.1371/journal.ppat.1003232.
56. Nanbo, A.; Maruyama, J.; Imai, M.; Ujie, M.; Fujioka, Y.; Nishide, S.; Takada, A.; Ohba, Y.; Kawaoka, Y. Ebola virus requires a host scramblase for externalization of phosphatidylserine on the surface of viral particles. *PLoS Pathog* **2018**, *14*, e1006848, doi:10.1371/journal.ppat.1006848.
57. Cheshenko, N.; Pierce, C.; Herold, B.C. Herpes simplex viruses activate phospholipid scramblase to redistribute phosphatidylserines and Akt to the outer leaflet of the plasma membrane and promote viral entry. *PLoS Pathog* **2018**, *14*, e1006766, doi:10.1371/journal.ppat.1006766.
58. Das, A.; Maury, W.; Lemon, S.M. TIM1 (HAVCR1): an Essential "Receptor" or an "Accessory Attachment Factor" for Hepatitis A Virus? *J Virol* **2019**, *93*, e01793-18, /jvi/93/11/JVI.01793-18.atom, doi:10.1128/JVI.01793-18.

Peer-reviewed version available at *Metabolites* **2020**, *10*, 356; doi:10.3390/metabo10090356

59. van den Bout, I.; Divecha, N. PIP5K-driven PtdIns(4,5)P2 synthesis: regulation and cellular functions. *Journal of Cell Science* **2009**, *122*, 3837–3850, doi:10.1242/jcs.056127.
60. Vicinanza, M.; D'Angelo, G.; Di Campli, A.; De Matteis, M.A. Function and dysfunction of the PI system in membrane trafficking. *EMBO J* **2008**, *27*, 2457–2470, doi:10.1038/emboj.2008.169.
61. Rocha-Perugini, V.; Gordon-Alonso, M.; Sánchez-Madrid, F. PIP2: choreographer of actin-adaptor proteins in the HIV-1 dance. *Trends in Microbiology* **2014**, *22*, 379–388, doi:10.1016/j.tim.2014.03.009.
62. Mücksch, F.; Laketa, V.; Müller, B.; Schultz, C.; Kräusslich, H.-G. Synchronized HIV assembly by tunable PIP2 changes reveals PIP2 requirement for stable Gag anchoring. *eLife* **2017**, *6*, e25287, doi:10.7554/eLife.25287.
63. Mücksch, F.; Citir, M.; Lüchtenborg, C.; Glass, B.; Traynor-Kaplan, A.; Schultz, C.; Brügger, B.; Kräusslich, H.-G. Quantification of phosphoinositides reveals strong enrichment of PIP2 in HIV-1 compared to producer cell membranes. *Sci Rep* **2019**, *9*, 17661, doi:10.1038/s41598-019-53939-z.
64. Mücksch, F.; Citir, M.; Lüchtenborg, C.; Glass, B.; Traynor-Kaplan, A.; Schultz, C.; Brügger, B.; Kräusslich, H.-G. Quantification of phosphoinositides reveals strong enrichment of PIP2 in HIV-1 compared to producer cell membranes. *Sci Rep* **2019**, *9*, 17661, doi:10.1038/s41598-019-53939-z.
65. Wijesinghe, K.J.; Stahelin, R.V. Investigation of the Lipid Binding Properties of the Marburg Virus Matrix Protein VP40. *J. Virol.* **2016**, *90*, 3074–3085, doi:10.1128/JVI.02607-15.
66. Gonzales, B.; de Rocquigny, H.; Beziau, A.; Durand, S.; Burlaud-Gaillard, J.; Lefebvre, A.; Krull, S.; Emond, P.; Brand, D.; Piver, E. Type I Phosphatidylinositol-4-Phosphate 5-Kinases α and γ Play a Key Role in Targeting HIV-1 Pr55 ^{Gag} to the Plasma Membrane. *J Virol* **2020**, *94*, e00189-20, /jvi/94/14/JVI.00189-20.atom, doi:10.1128/JVI.00189-20.
67. Diehl, N.; Schaal, H. Make Yourself at Home: Viral Hijacking of the PI3K/Akt Signaling Pathway. *Viruses* **2013**, *5*, 3192–3212, doi:10.3390/v5123192.
68. Wu, S.-X.; Chen, W.-N.; Jing, Z.-T.; Liu, W.; Lin, X.-J.; Lin, X. Hepatitis B Spliced Protein (HBSP) Suppresses Fas-Mediated Hepatocyte Apoptosis via Activation of PI3K/Akt Signaling. *J Virol* **2018**, *92*, e01273-18, /jvi/92/23/e01273-18.atom, doi:10.1128/JVI.01273-18.
69. Heaton, N.S.; Randall, G. Multifaceted roles for lipids in viral infection. *Trends in Microbiology* **2011**, *19*, 368–375, doi:10.1016/j.tim.2011.03.007.
70. Harak, C.; Lohmann, V. Ultrastructure of the replication sites of positive-strand RNA viruses. *Virology* **2015**, *479–480*, 418–433, doi:10.1016/j.virol.2015.02.029.
71. Neuman, B.W.; Angelini, M.M.; Buchmeier, M.J. Does form meet function in the coronavirus replicative organelle? *Trends in Microbiology* **2014**, *22*, 642–647, doi:10.1016/j.tim.2014.06.003.
72. Paul, D.; Hoppe, S.; Saher, G.; Krijnse-Locker, J.; Bartenschlager, R. Morphological and Biochemical Characterization of the Membranous Hepatitis C Virus Replication Compartment. *Journal of Virology* **2013**, *87*, 10612–10627, doi:10.1128/JVI.01370-13.
73. Knoops, K.; Kikkert, M.; Worm, S.H.E. van den; Zevenhoven-Dobbe, J.C.; van der Meer, Y.; Koster, A.J.; Mommaas, A.M.; Snijder, E.J. SARS-Coronavirus Replication Is Supported by a Reticulovesicular Network of Modified Endoplasmic Reticulum. *PLoS Biol* **2008**, *6*, e226, doi:10.1371/journal.pbio.0060226.
74. Netherton, C.L.; Wileman, T. Virus factories, double membrane vesicles and viroplasm generated in animal cells. *Current Opinion in Virology* **2011**, *1*, 381–387, doi:10.1016/j.coviro.2011.09.008.
75. Romero-Brey, I.; Merz, A.; Chiramel, A.; Lee, J.-Y.; Chlanda, P.; Haselman, U.; Santarella-Mellwig, R.; Habermann, A.; Hoppe, S.; Kallis, S.; et al. Three-Dimensional Architecture and Biogenesis of Membrane Structures Associated with Hepatitis C Virus Replication. *PLoS Pathog* **2012**, *8*, e1003056, doi:10.1371/journal.ppat.1003056.

Peer-reviewed version available at Metabolites 2020, 10, 356; doi:10.3390/metabo10090356

76. Egger, D.; Wölk, B.; Gosert, R.; Bianchi, L.; Blum, H.E.; Moradpour, D.; Bienz, K. Expression of Hepatitis C Virus Proteins Induces Distinct Membrane Alterations Including a Candidate Viral Replication Complex. *JVI* **2002**, *76*, 5974–5984, doi:10.1128/JVI.76.12.5974-5984.2002.
77. Wang, H.; Tai, A. Mechanisms of Cellular Membrane Reorganization to Support Hepatitis C Virus Replication. *Viruses* **2016**, *8*, 142, doi:10.3390/v8050142.
78. Angelini, M.M.; Akhlaghpour, M.; Neuman, B.W.; Buchmeier, M.J. Severe Acute Respiratory Syndrome Coronavirus Nonstructural Proteins 3, 4, and 6 Induce Double-Membrane Vesicles. *mBio* **2013**, *4*, e00524-13, doi:10.1128/mBio.00524-13.
79. Brugger, B.; Glass, B.; Haberkant, P.; Leibrecht, I.; Wieland, F.T.; Krausslich, H.-G. The HIV lipidome: A raft with an unusual composition. *Proceedings of the National Academy of Sciences* **2006**, *103*, 2641–2646, doi:10.1073/pnas.0511136103.
80. Martin-Acebes, M.A.; Merino-Ramos, T.; Blazquez, A.-B.; Casas, J.; Escribano-Romero, E.; Sobrino, F.; Saiz, J.-C. The Composition of West Nile Virus Lipid Envelope Unveils a Role of Sphingolipid Metabolism in Flavivirus Biogenesis. *Journal of Virology* **2014**, *88*, 12041–12054, doi:10.1128/JVI.02061-14.
81. Wang, H.; Perry, J.W.; Lauring, A.S.; Neddermann, P.; De Francesco, R.; Tai, A.W. Oxysterol-Binding Protein Is a Phosphatidylinositol 4-Kinase Effector Required for HCV Replication Membrane Integrity and Cholesterol Trafficking. *Gastroenterology* **2014**, *146*, 1373-1385.e11, doi:10.1053/j.gastro.2014.02.002.
82. Khan, I.; Katikaneni, D.S.; Han, Q.; Sanchez-Felipe, L.; Hanada, K.; Ambrose, R.L.; Mackenzie, J.M.; Konan, K.V. Modulation of Hepatitis C Virus Genome Replication by Glycosphingolipids and Four-Phosphate Adaptor Protein 2. *Journal of Virology* **2014**, *88*, 12276–12295, doi:10.1128/JVI.00970-14.
83. Delang, L.; Harak, C.; Benkheil, M.; Khan, H.; Leyssen, P.; Andrews, M.; Lohmann, V.; Neyts, J. PI4KIII inhibitor enviroxime impedes the replication of the hepatitis C virus by inhibiting PI3 kinases. *Journal of Antimicrobial Chemotherapy* **2018**, doi:10.1093/jac/dky327.
84. Arita, M.; Kojima, H.; Nagano, T.; Okabe, T.; Wakita, T.; Shimizu, H. Phosphatidylinositol 4-kinase III beta is a target of enviroxime-like compounds for antipoliovirus activity. *J. Virol.* **2011**, *85*, 2364–2372, doi:10.1128/JVI.02249-10.
85. Martín-Acebes, M.A.; Blázquez, A.-B.; Jiménez de Oya, N.; Escribano-Romero, E.; Saiz, J.-C. West Nile Virus Replication Requires Fatty Acid Synthesis but Is Independent on Phosphatidylinositol-4-Phosphate Lipids. *PLoS ONE* **2011**, *6*, e24970, doi:10.1371/journal.pone.0024970.
86. Yang, N.; Ma, P.; Lang, J.; Zhang, Y.; Deng, J.; Ju, X.; Zhang, G.; Jiang, C. Phosphatidylinositol 4-Kinase IIIβ Is Required for Severe Acute Respiratory Syndrome Coronavirus Spike-mediated Cell Entry. *J. Biol. Chem.* **2012**, *287*, 8457–8467, doi:10.1074/jbc.M111.312561.
87. de Wilde, A.H.; Wannee, K.F.; Scholte, F.E.M.; Goeman, J.J.; ten Dijke, P.; Snijder, E.J.; Kikkert, M.; van Hemert, M.J. A Kinome-Wide Small Interfering RNA Screen Identifies Proviral and Antiviral Host Factors in Severe Acute Respiratory Syndrome Coronavirus Replication, Including Double-Stranded RNA-Activated Protein Kinase and Early Secretory Pathway Proteins. *J. Virol.* **2015**, *89*, 8318–8333, doi:10.1128/JVI.01029-15.
88. de Wilde, A.H.; Snijder, E.J.; Kikkert, M.; van Hemert, M.J. Host Factors in Coronavirus Replication. In *Roles of Host Gene and Non-coding RNA Expression in Virus Infection*; Tripp, R.A., Tompkins, S.M., Eds.; Current Topics in Microbiology and Immunology; Springer International Publishing: Cham, 2017; Vol. 419, pp. 1–42 ISBN 978-3-030-05368-0.
89. Cottam, E.M.; Maier, H.J.; Manifava, M.; Vaux, L.C.; Chandra-Schoenfelder, P.; Gerner, W.; Britton, P.; Ktistakis, N.T.; Wileman, T. Coronavirus nsp6 proteins generate autophagosomes from the endoplasmic reticulum via an omegasome intermediate. *Autophagy* **2011**, *7*, 1335–1347, doi:10.4161/auto.7.11.16642.

Peer-reviewed version available at Metabolites 2020, 10, 356; doi:10.3390/metabo10090356

90. Horton, J.D.; Goldstein, J.L.; Brown, M.S. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* **2002**, *109*, 1125–1131, doi:10.1172/JCI15593.
91. Yuan, S.; Chu, H.; Chan, J.F.-W.; Ye, Z.-W.; Wen, L.; Yan, B.; Lai, P.-M.; Tee, K.-M.; Huang, J.; Chen, D.; et al. SREBP-dependent lipidomic reprogramming as a broad-spectrum antiviral target. *Nat Commun* **2019**, *10*, 120, doi:10.1038/s41467-018-08015-x.
92. Diamond, D.L.; Syder, A.J.; Jacobs, J.M.; Sorensen, C.M.; Walters, K.-A.; Proll, S.C.; McDermott, J.E.; Gritsenko, M.A.; Zhang, Q.; Zhao, R.; et al. Temporal Proteome and Lipidome Profiles Reveal Hepatitis C Virus-Associated Reprogramming of Hepatocellular Metabolism and Bioenergetics. *PLoS Pathog* **2010**, *6*, e1000719, doi:10.1371/journal.ppat.1000719.
93. Wu, Q.; Zhou, L.; Sun, X.; Yan, Z.; Hu, C.; Wu, J.; Xu, L.; Li, X.; Liu, H.; Yin, P.; et al. Altered Lipid Metabolism in Recovered SARS Patients Twelve Years after Infection. *Sci Rep* **2017**, *7*, 9110, doi:10.1038/s41598-017-09536-z.
94. Vijay, R.; Hua, X.; Meyerholz, D.K.; Miki, Y.; Yamamoto, K.; Gelb, M.; Murakami, M.; Perlman, S. Critical role of phospholipase A2 group IID in age-related susceptibility to severe acute respiratory syndrome–CoV infection. *Journal of Experimental Medicine* **2015**, *212*, 1851–1868, doi:10.1084/jem.20150632.
95. Vijay, R.; Fehr, A.R.; Janowski, A.M.; Athmer, J.; Wheeler, D.L.; Grunewald, M.; Sompallae, R.; Kurup, S.P.; Meyerholz, D.K.; Sutterwala, F.S.; et al. Virus-induced inflammasome activation is suppressed by prostaglandin D_2 /DP1 signaling. *Proc Natl Acad Sci USA* **2017**, *114*, E5444–E5453, doi:10.1073/pnas.1704099114.
96. Tay, M.Z.; Poh, C.M.; Rénia, L.; MacAry, P.A.; Ng, L.F.P. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol* **2020**, *20*, 363–374, doi:10.1038/s41577-020-0311-8.
97. Yan, B.; Chu, H.; Yang, D.; Sze, K.-H.; Lai, P.-M.; Yuan, S.; Shuai, H.; Wang, Y.; Kao, R.Y.-T.; Chan, J.F.-W.; et al. Characterization of the Lipidomic Profile of Human Coronavirus-Infected Cells: Implications for Lipid Metabolism Remodeling upon Coronavirus Replication. *Viruses* **2019**, *11*, 73, doi:10.3390/v11010073.
98. Grant, W.B.; Lahore, H.; McDonnell, S.L.; Baggerly, C.A.; French, C.B.; Aliano, J.L.; Bhattoa, H.P. Evidence that Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths. *Nutrients* **2020**, *12*, 988, doi:10.3390/nu12040988.
99. Holick, M.F. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *The American Journal of Clinical Nutrition* **2004**, *80*, 1678S-1688S, doi:10.1093/ajcn/80.6.1678S.
100. Martínez-Moreno, J.; Hernandez, J.C.; Urcuqui-Inchima, S. Effect of high doses of vitamin D supplementation on dengue virus replication, Toll-like receptor expression, and cytokine profiles on dendritic cells. *Mol Cell Biochem* **2020**, *464*, 169–180, doi:10.1007/s11010-019-03658-w.
101. Nieva, J.L.; Madan, V.; Carrasco, L. Viroporins: structure and biological functions. *Nat Rev Microbiol* **2012**, *10*, 563–574, doi:10.1038/nrmicro2820.
102. Sze, C.; Tan, Y.-J. Viral Membrane Channels: Role and Function in the Virus Life Cycle. *Viruses* **2015**, *7*, 3261–3284, doi:10.3390/v7062771.