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Review

ZIKA virus entry mechanisms in human cells

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ABSTRACT

Zika virus (ZIKV) is an enveloped, mosquito-borne *Flavivirus*, which infects cells through clathrin-mediated endocytosis and fusion employing acidic endosomes. Cell entry is mostly mediated by the viral glycoprotein E, although incomplete particle maturation enables viral protein prM and anionic lipids present in the viral membrane to mediate this process. Incomplete proteolytic maturation results in a set of highly heterogeneous particles. These heterogeneous and dynamic infectious particles offer a variety of possible receptor interaction sites on their surfaces, thus contributing to the wide range of cells susceptible to ZIKV as well as to variation in tissue tropism. This review addresses recent advances in the understanding of ZIKV entry process into cells and put together fundamental questions about viral replication, maturation and host-cell interactions.

1. Introduction

Zika virus (ZIKV) is an arbovirus belonging to the *Flaviviridae* family, genus *Flavivirus*, closely related to several other viruses that cause disease globally, including Dengue (DENV), Yellow Fever (YFV), West Nile (WNV), Japanese Encephalitis (JEV) and Tick-borne Encephalitis Virus (TBEV) (Alam et al., 2017; Miner and Diamond, 2017; Musso et al., 2015; Wang et al., 2017a).

ZIKV was first isolated in 1947 from the serum of a sentinel Rhesus monkey at the Zika forest, in Uganda (Dick et al., 1952). The first evidence of human infection was based on the presence of neutralizing antibodies against ZIKV in 1952, and it was first linked to Zika disease in 1964 (Simpson, 1964). Nevertheless, little attention was paid to this virus until outbreaks were reported in Yap Island, Federated States of Micronesia, in 2007 (Duffy et al., 2009), and in French Polynesia, 2013 (Cao-Lormeau et al., 2014), where it was associated with Guillain-Barré Syndrome. During the outbreak in Brazil, in 2015, ZIKV was linked to neonatal microcephaly (ECDC, 2015; Schuler-faccini et al., 2016). To date, in Brazil, there were 16,735 suspected cases of changes in growth and development of children related to ZIKV infection, with 3267 (19,5%) confirmed cases (Brazil Ministry of Health, 2018). These severe clinical implications have prompted the scientific community to develop emergency interventions, since the disease pathogenic mechanism is not yet completely understood.

ZIKV genome has ~10.7 kb, encoding 3423 amino acids, with two flanking 5′ and 3′ noncoding regions and a single long structure encoding a polyprotein, which is cleaved into capsid protein (C), precursor protein (prM), envelope protein (E) and 7 non-structural proteins (NS): 50-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-30 (Dikhit et al., 2016; Göertz et al., 2017). Viral envelope protein E intermediates membrane fusion by receptor-mediated endocytosis mostly through C-type lectin receptors family from the host-cell (Dai et al., 2016; Kim et al., 2017a). Although, cellular lipid receptors, such as TIM (T-cell immunoglobulin mucin) and TAM (Receptor tyrosine kinases: **T**YRO3, **A**XL and **M**ER) receptor families (which recognize lipids in the viral membrane) showed to mediate ZIKV entry (Hastings et al., 2017; Liu et al., 2016; Meertens et al., 2017).

The molecules used by ZIKV in entry process depend on the pattern of proteins expressed in viral particle lattice and therefore appear to rely on the degree of particle maturation (Pierson and Diamond, 2013). Furthermore, cell type plays a major role in this mechanism (Perera-lecoin et al., 2014). Studies *in vivo* showed that cells of the reproductive tract (spermatogonia, sperm, Sertoli and Leydig cells) (Govero et al., 2016) and from cerebral cortex and hippocampus (Wang et al., 2017b) are permissive to ZIKV infection in mice. More importantly, using an *in vitro* approach, cell culture experiments demonstrated the ability of

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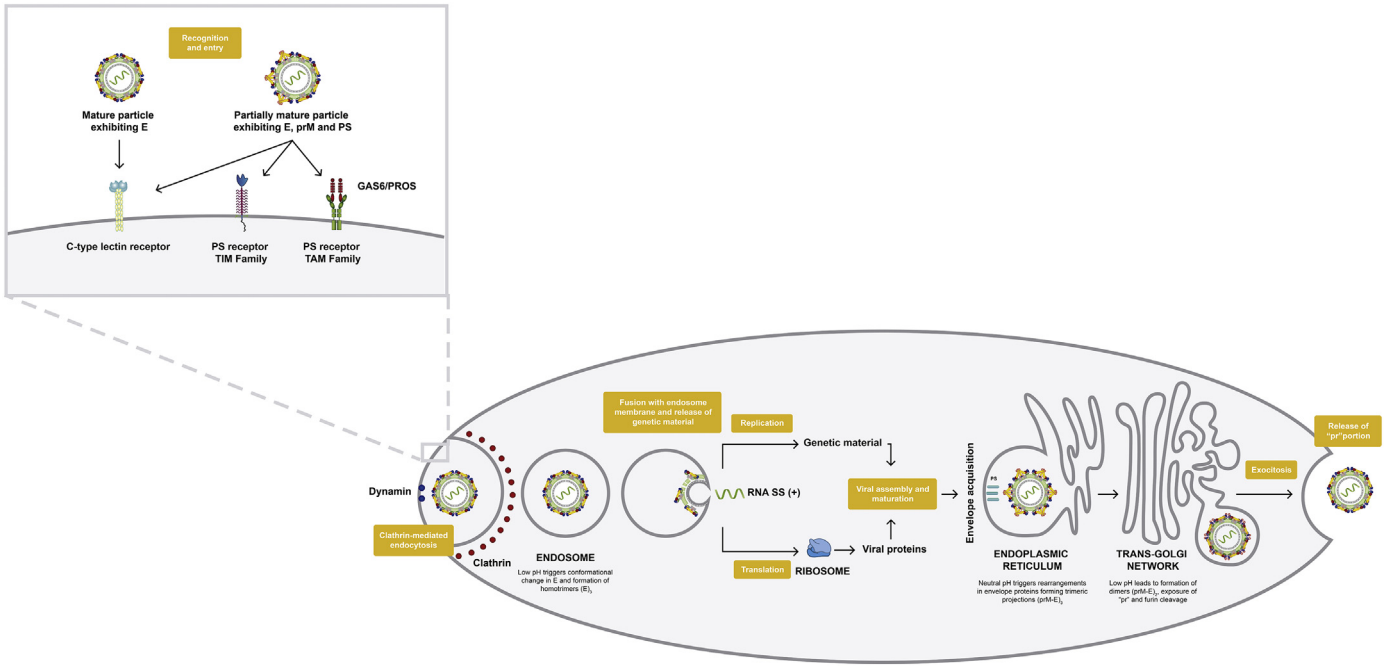

Fig. 1. ZIKV processes of attachment and entry, replication, translation, assembly, maturation and release from infected cells.

ZIKV to infect human cells, such as skin fibroblasts (Hamel et al., 2015), uterine fibroblasts (Chen et al., 2016), primary placental trophoblasts and Hofbauer cells (Aagaard et al., 2017; Quicke et al., 2016), endometrial stromal cells (Pagani et al., 2017), and neural progenitor cells (Dang et al., 2016; Tang et al., 2016). These findings hold for an extensive set of cells susceptible to ZIKV infection and therefore a robust number of receptors involved in viral entry process. In this review, we discuss viral maturation and heterogeneity in the context of ZIKV entry, highlighting recent advances in the understanding of molecular mechanisms involved in viral infectivity.

2. CLATHRIN-mediated endocytosis

The main mechanism by which *Flavivirus* infiltrate human host cells is clathrin-mediated endocytosis, which is followed by a change in envelope conformation, membrane fusion and release of the viral genome (Fig. 1) (Cruz-Oliveira et al., 2015; Hackett and Cherry, 2018). Clathrin-mediated endocytosis is a vesicular trafficking process that transports cargo molecules from the cell surface to its interior, and generally occurs at specialized sites, where a “coated pit” structure is assembled in order to concentrate surface proteins for internalization (Mousavi et al., 2004). This process (named after the most abundant protein found in coated pits: clathrin) is mostly involved in physiological processes, such as nutrient uptake, cell signaling and adhesion, *etc.*, however, the same mechanism has shown to be used by viruses, including ZIKV (Persaud et al., 2018), to enter cells (Kaksonen and Roux, 2018).

Initially, during viral infection, viral particles diffuse along the cell surface toward a pre-existing clathrin-coated pit. This implies that viruses either roll over distinct binding factors until they bind to the input receptor (located in clathrin hotspots on the cell surface), or that the initially formed virus-receptor complex is transported to a pre-existing clathrin-coated pit (Smit et al., 2011). Subsequently, the clathrin-coated pit evolves and invagination at the plasma membrane is closed by excision of the dynamin-mediated membrane to form a clathrin-coated vesicle. This vesicle is transported away from the plasma membrane, and then the clathrin coating is released from it. After clathrin-mediated uptake, the endocytic vesicle carrying the virus is delivered to the initial endosomes, which mature into late endosomes, and then the viral membrane fuses with the endosome membrane so viral RNA is released into the cytoplasm (Mercer et al., 2010). It is important to note that membrane fusion on the host cell depends on the pH of the viral membrane and therefore may vary between different strains (Chu and Ng, 2004; Cruz-Oliveira et al., 2015).

3. Attachment factors and entry receptors

It is likely that receptor binding and recognition is a process where multiple molecules are used in combination for infectious entry (Kaufmann and Rossmann, 2012; Kim et al., 2017a; Mercer et al., 2010). The surface of ZIKV mature particle is typically covered by homodimers of protein E $(E)_2$, arranged in an icosahedral format (Sirohi et al., 2016). Individual interactions with human receptors are generally weak, but contact with multiple receptors makes avidity higher and the bind becomes stronger (Mercer and Helenius, 2010). It is important to distinguish entry receptors - interactions that result in virus uptake - from attachment factors, which will only retain viral particles on cell surface until there is interaction with an entry receptor (Kim et al., 2017a). Generally, receptors promote endocytosis and accompany the virus into the cell (Kaufmann and Rossmann, 2012). Therefore, entry starts with binding to attachment factors, followed by associations with one or more receptors.

The most commonly encountered attachment factors are negatively charged glycosaminoglycans (GAGs), such as heparan sulfate and chondroitin sulfate (Kim et al., 2017a), which can be utilized by several flaviviruses, including DENV (Hilgard and Stockhert, 2000; Thepparit et al., 2004), WNV (Lee et al., 2004), JEV (Lee et al., 2004; Su et al., 2001) and TBEV (Kroschewski et al., 2003), as low-affinity attachment factors that concentrate the virus on the cell surface. Binding to these negatively charged polysaccharides is usually electrostatic and relatively nonspecific (Mercer et al., 2010). A surface plasmon resonance analysis that explored binding between GAGs prepared from placenta and protein E suggested that ZIKV may utilized GAGs as attachment factors for host cell entry, as other pathogenic flaviviruses (Kim et al., 2017b).

ZIKV entry into target cells is mostly mediated by interaction of N-glycans conjugated to protein E with cell surface receptors of the host cell (Hasan et al., 2017; Heinz and Stiasny, 2017). Protein E is the

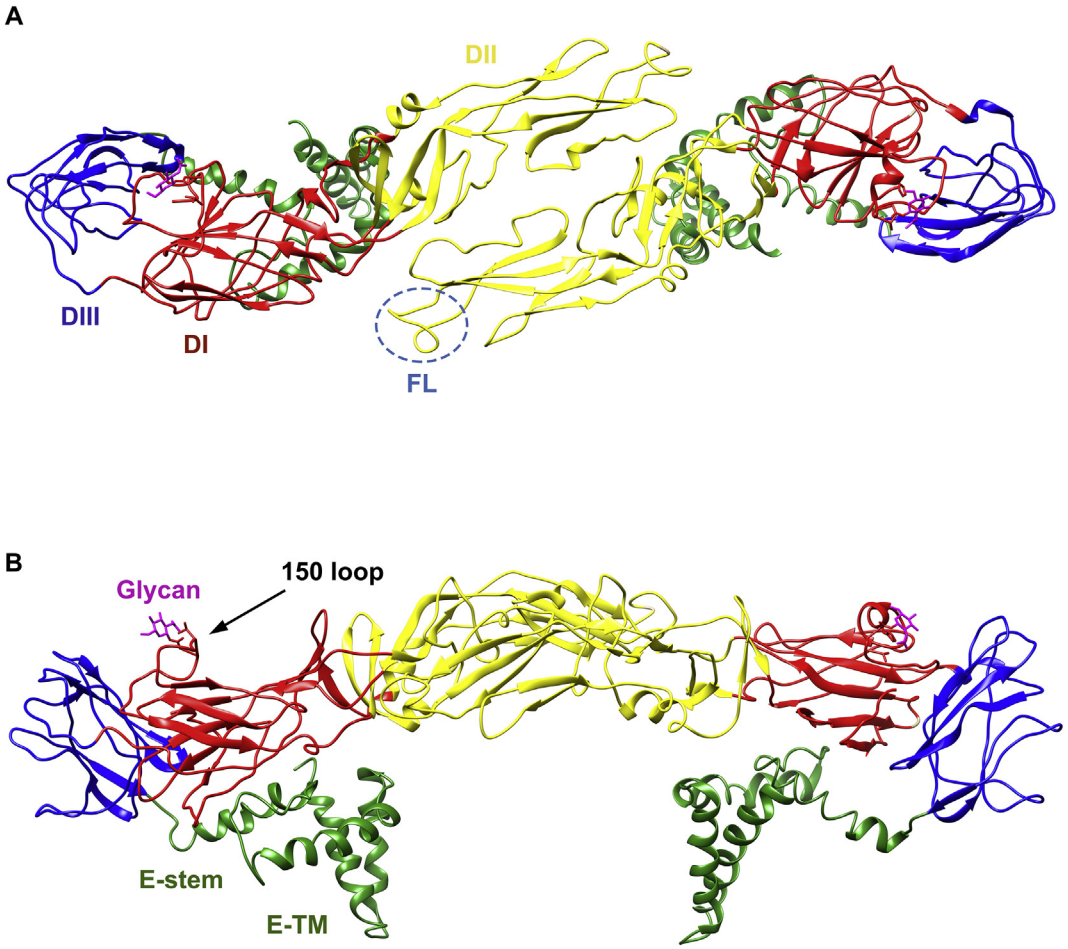

Fig. 2. Structure of ZIKV E protein dimers (PDB id code: 5IZ7). (A) E protein domains I (red), II (yellow) and III (blue). Fusion loop (FL) is evidenced in the dotted circle. (B) Stem-transmembrane domain that anchors the protein into the membrane is shown in green. The glycosylation site (with the glycan, in pink) is found in the 150 loop region. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

largest viral surface protein, being involved in membrane binding and fusion processes (Hasan et al., 2017; Shi and Gao, 2017). In ZIKV, protein E is N-glycosylated after the translation process at position Asn154 (asparagine, at position 154) within a highly conserved N-X-T/S sequence glycosylation motif (in which N is asparagine, T is threonine and X is any standard amino acid, except proline) at positions 154–156, indicating the biological importance of this modification (Fontes-garfias et al., 2017). However, other *Flavivirus* do not exhibit such glycosylation, suggesting that protein E function can be achieved without N-glycan (Adams et al., 1995; Beasley et al., 2004).

Similar to other *Flavivirus*, ZIKV protein E has four domains: the stem-transmembrane domain, which is responsible for membrane anchoring; and domains I, II and III, which constitute the predominantly β-strand surface part of the protein (Heinz and Stiasny, 2017; Ye et al., 2016).

Domain I (DI) of protein E acts as a bridge between domains II (DII) and III (DIII) (Fig. 2a). The tip of DII contains the fusion loop (FL), which interacts with the host membrane during membrane fusion and is the most conserved structural element in this protein among all *Flavivirus* (Heinz and Stiasny, 2012). FL is responsible for the broad antibody cross-reactivity observed among all *Flavivirus* and plays an important role as an antigenic site that contributes to antibody-mediated enhancement of infection. A study based on chemical cross-linking and immunoassays demonstrated that monoclonal antibodies recognize epitopes at the interface of the dimeric envelope protein E, cause dimer dissociation and lead to the exposure of FL in TBEV and DENV (Haslwanter et al., 2017). Under usual infection conditions, this process is only triggered after the virus contact with the acidic pH of endosomes, resulting in membrane fusion through the interaction of FL with the endosomal membrane (Mercer et al., 2010; Smit et al., 2011). This exposure of FL at neutral pH may mediate attachment of the virus to the plasma membrane, thereby increasing viral infectivity.

Receptor-binding site is localized on DIII, being of great importance in the fusion process. Curiously, ZIKV has a single glycosylation site (Asn154) on DI of protein E (Fig. 2b), which protrudes from the surface, because ZIKV *E*-DI has a longer “150 loop” (residues 145–160) compared to other flaviviruses. This 150 loop region varies not only between ZIKV strains but also in other *Flavivirus*, suggesting that variations in this region influence viral infectivity (Shi and Gao, 2017).

Virions are not static structures and undergo concerted fluctuations at equilibrium, a phenomenon referred to as viral “breathing” (Lewis et al., 1998). More recent evidence indicates that metastability of the E dimers gives rise to a dynamic motion, in which they transiently expose otherwise buried surfaces (Kuhn et al., 2015). These structural dynamics of the surface of the virus transiently expose the E-sites that would be inaccessible in a static viral particle with a closed envelope of 90 E dimers in a fishbone arrangement, as determined by cryo EM (Kostyuchenko et al., 2016). Dynamics of fully and partially mature particles may thus facilitate interactions with cellular attachment factors and entry receptors.

4. C-type lectin receptors

The innate immune system is responsible for the body's first line of

defense against microbial attack and it is induced by the recognition of microbial components, known as pathogen-associated molecular patterns (PAMPs). Such recognition initiates signaling cascades that induce the intracellular innate immune defenses and the inflammatory response which facilitates the development of the acquired immune response (Hoving et al., 2014; Kell and Gale Jr, 2015). Viruses are recognized by human pattern recognition receptors (PRRs) through their nucleic acids, such as double (dsRNA), single stranded-RNA (ssRNA), and DNA, although envelope glycoproteins on surface of viral particle can also be recognized as PAMPs (Hoving et al., 2014).

C-type lectin receptors (CLRs) comprise a large family of carbohydrate receptors, which bind through one or more carbohydrate recognition domains (CRDs), and is divided into 17 groups taking into account features such as phylogeny and structure (Zelensky and Gready, 2005). Several members of this family are highly expressed in myeloid cells, including monocytes, macrophages and dendritic cells, thus playing a central role in the activation of the host immune system (Kim et al., 2017a).

CLRs recognize carbohydrate profiles in pathogens and act as PPRs for the internalization of these agents, directing them to endosomes, initiating the process of antigen presentation and elimination of the pathogen (Cambi et al., 2005). The most implicated CLRs in *Flavivirus* entry are DC-SIGN (Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) (Hacker et al., 2009; Lozach et al., 2005; Navarro-Sanchez et al., 2003; Tassaneetrithep et al., 2003), L-SIGN (Liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin) (Navarro-Sanchez et al., 2003; Wang et al., 2016), MMR (Macrophage mannose receptor) (Miller et al., 2008) e CLEC5A (C-type lectin member 5A) (Chen et al., 2008).

DC-SIGN showed to permit ZIKV entry into cells (Hamel et al., 2015). Additionally, *in vitro* analysis carried out with different cell lineages revealed similar results with DENV (Alen et al., 2009; Hacker et al., 2009; Lozach et al., 2005; Tassaneetrithep et al., 2003), WNV (Davis et al., 2006b; Martina et al., 2008) and JEV (Shimojima et al., 2014), whereas L-SIGN demonstrated to enable cell entry only for WNV (Davis et al., 2006b) and JEV (Shimojima et al., 2014). Studies have identified a single nucleotide polymorphism in the promoter sequence of DC-SIGN gene (CD209) that is strongly associated with predisposition to dengue hemorrhagic fever (rs4804803) (Wang et al., 2011) and severe forms of tick-borne encephalitis (rs2287886) (Barkhash et al., 2012), which reinforces the involvement of this receptor in *Flavivirus* tropism.

DC-SIGN and its close homologue L-SIGN are group II (calcium-dependent with single CRD) transmembrane C-type lectins and interact through their CRD domains with carbohydrates bound to viral protein E (Cruz-Oliveira et al., 2015; Zelensky and Gready, 2005). Their extracellular domains share common structural motifs, including an extended neck composed of tandem repeats of a highly conserved 23-amino acid sequence, which is followed by a CRD that binds mannose-rich glycans and plays an important role in entry mechanisms of *Flavivirus* into myeloid cells (Khoo et al., 2008). DC-SIGN is highly expressed in some subsets of immature macrophages and dendritic cells, suggesting a possible facilitation in viral dissemination (Hamel et al., 2015; Kay et al., 2012; Smit et al., 2011). Contrarily, L-SIGN expression is restricted to sinusoidal endothelial cells of the liver and endothelial cells of lymph nodes (Tassaneetrithep et al., 2003), however its role during the course of infection has not yet been established (Perera-lecoin et al., 2014).

MMR is a group VI (calcium-dependent with multiple CRDs) CLR that has been proposed to be a functional entry receptor for *Flavivirus* (Hafizi and Dahlbäck, 2006). Unlike DC-SIGN and L-SIGN, MMR has several CRD domains and a cysteine-rich domain at the end of its extracellular domain that is capable of interacting with sulfated sugars (Hamel et al., 2015; Martinez-pomares, 2012). MMR is essentially expressed in macrophages, but can also be found in lymph nodes and endothelial cells of the liver, in kidney cells and in some dendritic cells populations (Martinez-pomares, 2012) - all relevant to *Flavivirus* infection. This receptor demonstrated to bind protein E of four DENV serotypes and was proposed as a DENV internalization receptor in human primary macrophages, since anti-MMR polyclonal antibodies inhibit virus infection (Dejnirattisai et al., 2016). CLEC5A is a group V CLR (calcium-independent with single CRD) (Kim et al., 2017a), which is expressed in monocytes and macrophages and has showed to mediate the entry of DENV (Chen et al., 2008) and JEV (Chen et al., 2012) into cells. Even though strong evidence link L-SIGN, MMR and CLEC5A *to Flavivirus entry,* we did not find in literature studies relating these receptors particularly in ZIKV entry process. Nevertheless, considering the similarity between these viruses, it is possible that ZIKV explores multiple CLRs for entry and greater viral spread.

5. Entry mediated by negatively charged lipids

Previous studies indicate that the entry process of flaviviruses, including ZIKV, may be mediated by interactions that do not involve protein E, but occur between negatively charged lipids, such as phosphatidylserine (PS), present in the viral membrane (Cruz-Oliveira et al., 2015; Hafizi and Dahlbäck, 2006; Hamel et al., 2015; Heinz and Stiasny, 2017; Meertens et al., 2012; Perera-lecoin et al., 2014). These anionic lipid cell receptors belong to two distinct families of transmembrane phosphatidylserine receptors: TIM (TIM1, TIM3 and TIM4) and TAM (**TY**RO3, **A**XL and **M**ER) (Kay et al., 2012; Lemke and Rothlin, 2010; Meertens et al., 2012).

On the contrary of TIM receptors, TAM receptors do not bind directly to PS, but indirectly, through a process that requires the presence of GAS6 (Growth-arrest-specific 6) or PROS (Protein S), which act as bridging molecules (Hafizi and Dahlbäck, 2006; Lemke and Rothlin, 2010; Meertens et al., 2012). The physiological function of these receptors is to recognize negatively charged lipids in apoptotic cells and trigger endocytosis by phagocytic cells (Fairn et al., 2011; Mercer and Helenius, 2010). This process of “kidnapping” was previously identified in a large number of viruses and is called “apoptotic mimicry” (Amara and Mercer, 2015).

As *Flavivirus* sprouts in the lumen of the endoplasmic reticulum (ER) during morphogenesis, the viral membrane ends up reflecting the ER membrane composition, which has PS in its luminal leaflet. The plasma membrane of living cells normally does not contain negatively charged lipids in the external leaflet due to the presence of specific enzymes called “lipid flipases” that assure the asymmetry of the plasmatic membrane by keeping these lipids only in the internal leaflet (Fairn et al., 2011; Kay et al., 2012).

The role of phosphatidylserine receptors in the entry of *Flavivirus* into cells was first identified in DENV studies, but several evidences suggest that ZIKV can also use apoptotic mimicry to infect different human tissues, including skin cells (Hamel et al., 2015), endothelial cells (Liu et al., 2016), neural cells (Retallack et al., 2016; Tang et al., 2016; Wells et al., 2016), and placenta cells (Tabata et al., 2016). A study carried out with a TAM-knockout mice demonstrated that these molecules are not necessary for ZIKV infectivity and virus replication has not been affected in the spleen, placenta, vagina and brain (Hastings et al., 2017). The data corroborate with previous observations (Govero et al., 2016) and suggest that there may be redundancy of ZIKV input receptors in cells.

Interestingly, a recent case series where placental tissues (from women who had laboratory-confirmed ZIKV during pregnancy) were analyzed showed that Hofbauer cells sustain the presence of the virus in the placenta until birth and may provide a viral source for continued infection (Noronha et al., 2018). Additionally, a sophisticated model of uterine-placental route of transmission was suggested by Tabata et al., 2016. This model proposes that ZIKV may disseminate by infecting invasive cytotrophoblasts (CTBs), which leave from chorionic villus of the placenta and surpass uterine wall to remodel uterine arteries. Once in contact with maternal infected blood, CTBs get infected and may

infect trophoblast progenitor cells in the chorionic membrane and then amniotic epithelial cells in the amniotic membrane. Thus, virions released into amniotic fluid may infect cells from the fetus. This elegant work also reported that TIM1 plays a critical role in the uterine-placental interface once its inhibitor blocked ZIKV infection *in vitro*. These data reinforce the involvement of phosphatidylserine receptors in ZIKV entry mechanism and raise questions on the possibility of an underlying cell-to-cell transmission mechanism. Further investigations on virological synapses are required to elucidate ZIKV infectivity processes.

6. Role of particle maturation in ZIKV infectivity and prM as a putative entry receptor

Maturation of *Flavivirus* from immature particles (C, prM and E) to mature form (C, M and E) occurs during viral exocytosis of an infected cell (Dowd et al., 2014). During entry into the host cell, the acidic endosomal environment triggers an irreversible conformational change in protein E and a transition from a homodimer $(E)_2$ to a homotrimer $(E)_3$ formation - the final, lowest-energy conformation of E, which leads to membrane fusion and release of viral genetic material and further replication and translation processes (Yu et al., 2014). In ER, during particle assembly process, the immature newly assembled particle exhibits a spiny surface, with 180 prM-E heterodimers associated into 60 trimeric projections $(prM\text{-}E)_3$ (Fig. 3a). During viral maturation, the low pH within the trans-Golgi network induces a reorganization of $(prM\text{-}E)_3$ trimeric projections into 90 dimers $(prM\text{-}E)_2$ (Yu et al., 2014). This structural rearrangement exposes the cleavage site of prM to be digested by the host protease, furin (Heinz and Stiasny, 2012). The “pr” portion cleaved from prM remains associated with the virus until the cell is released, where it finally dissociates, due to the neutral pH of the extracellular medium (Zhang et al., 2007).

Fully mature particles incorporate proteins E $(E)_2$ and M $(M)_2$ as 90 homodimers each (Fig. 3b), arranged in closely packed protein shell with a herringbone pattern, and lacking non-cleaved prM protein (Yu et al., 2014). Several lines of evidence indicate that cleavage of prM may be inefficient and that infectious viral particles released from the cells may have non-cleaved prM. The resulting particles, therefore, exhibit a heterogeneous architecture, exposing both mature and immature particle structure (Fig. 3c) (Rey et al., 2017; Yu et al., 2014).

Heterogeneity of viral particles appears to be necessary for the use of apoptotic mimicry in the entry process of ZIKV into cells because the viral membrane containing PS would not be accessible in the mature viruses (Smit et al., 2011). We did not find any reports in literature about *in vitro* assays that exploited the maturation status of ZIKV particles to their phosphatidylserine receptor-mediated entry ability. It must be noted that different *Flavivirus*, or even different strains of the same virus, are likely to demonstrate different degrees of membrane exposure and may therefore differ in the use of entry lipid receptors.

The release of partially mature but infectious particles suggests that prM or prM-E complex exposed on the surface of these particles could mediate viral entry process (Heinz and Stiasny, 2017). DENV has previously shown to be particularly rich in partially mature particles, since a sub-optimal furin cleavage motif in prM has evolved, exhibiting a conserved amino acid residue at the P3 cleavage position, which has shown to have a negative effect on the efficiency of furin cleavage (Junjhon et al., 2008). Thus, partial maturation seems to be important in the maintenance of viral ecological cycle. Another study conducted with WNV has demonstrated that N-glycosylated prM can mediate viral entry into cells *via* DC-SIGN (Davis et al., 2006a). A single mutation in prM (serine to asparagine substitution at position 139) has been associated with the ability of ZIKV to infect cells (Yuan et al., 2017), suggesting that prM could also act as an entry receptor.

6.1. Inhibition of ZIKV entry

Since ZIKV infection has become a serious public health issue, many efforts have been made to develop an anti-ZIKV drug. In this regard, many molecules demonstrated to be promising inhibitors of different stages of ZIKV's life cycle, such as viral entry, replication, maturation and release of infectious particles, although until date there is still no specific drug or vaccine against ZIKV infection (Munjal et al., 2017).

Concerning ZIKV entry, some molecules are known to block early viral mechanisms: by interfering with receptor binding or inhibiting internalization by endocytosis. Studies *in vitro* performed on different cell lines showed that curcumin (Mounce et al., 2017), nanchangmycin (Rausch et al., 2017), ZINC33683341 and ZINC49605556 (Sandun et al., 2016) molecules inhibit viral entry by blocking receptor binding, while compounds such as chloroquine (Li et al., 2017b; Shiryaev et al., 2017), suramin (Albulescu et al., 2017; Tan et al., 2017) and 25-hydroxycholesterol (Li et al., 2017a) demonstrated to inhibit ZIKV internalization *in vitro* and *in vivo*. Chloroquine's is controversial about its mechanism of action, whether if it is in viral entry or autophagic flux. Several tests *in vitro* and *in vivo* were carried out on both perspectives and appear to evidence a double mechanism for this drug (Cao et al., 2017; Li et al., 2017b; Liang et al., 2016; Shiryaev et al., 2017; Zhang et al., 2017). Apart from blocking receptor binding by targeting AXL, nanchangmycin also seems to play a double role by inhibiting clathrin-mediated endocytosis (Rausch et al., 2017). Further, arbitol, a well-known antiviral of broad-spectrum, showed to prevent an early step of ZIKV's lifecycle *in vitro*, although its precise mechanism of action is not well known yet (Fink et al., 2018). Epigallocatechin gallate (EGCG), a compound largely present in green tea, demonstrated to considerably reduce ZIKV infectivity in Vero cells, however its action seems to be viricidal by directly interacting with ZIKV's envelope (Carneiro et al., 2016). Additional tests with these drugs are clearly necessary for the development of a safe and effective treatment against ZIKV. We summarized the mechanisms of these anti-ZIKV drugs in Table 1.

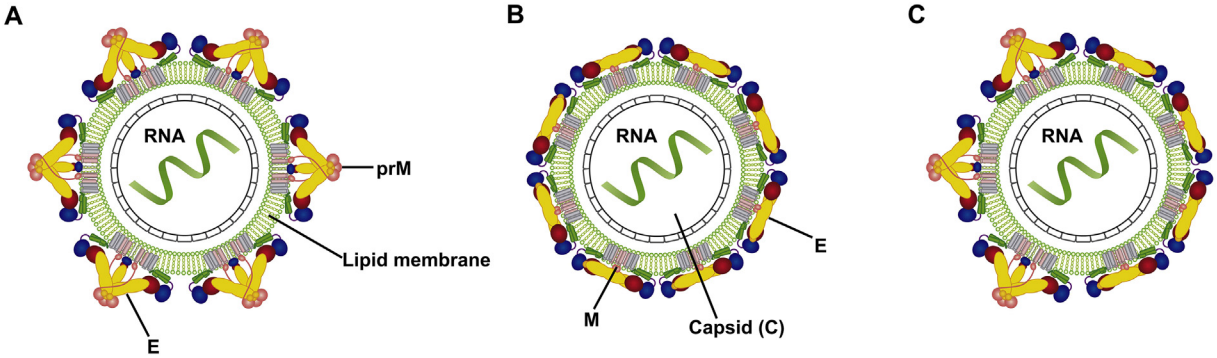

Fig. 3. Viral particle possible structures. (A) Immature particle displaying trimeric projections $(prM\text{-}E)_3$ due to neutral pH of endoplasmic reticulum. (B) Mature particle displaying E $(E)_2$ and M $(M)_2$ homodimers after complete cleavage by furin and secretion from host cells. (C) Heterogeneous particle structure.

Table 1
List of potential inhibitors of early stage in ZIKV infection.

Compound	Study model	Effects	Putative mechanism of action	Reference
Arbidol	*In vitro*	Inhibits viral infection of primary vaginal (HVE2) and cervical epithelial cells (ENDO and ECTO cells) and reduces the production of progeny virions	Inhibits an early step in viral lifecycle	(Fink et al., 2018)
Chloroquine	*In vitro/In vivo*	Inhibits viral infection *in vitro* (BHK-21, Huh7 and Vero cells) and attenuates ZIKV-associated morbidity and mortality in mice	Blocks viral internalization	(Li et al., 2017b)
Curcumin	*In vitro*	Reduces infectivity in HeLa, BHK-21 and Vero cells	Inhibits viral entry by interfering with the binding of protein E	(Mounce et al., 2017)
EGCG	*In vitro*	Reduces infectivity in Vero cells	Viricidal effect probably by direct interaction of the drug with lipid envelope, leading to a subsequent destruction of the virus particle	(Carneiro et al., 2016)
Nanchangmycin	*In vitro*	Blocks ZIKV replication in U2OS cells	Targets AXL receptors and blocks clathrin-mediated endocytosis	(Rausch et al., 2017)
Suramin	*In vitro*	Inhibits viral attachment and the release of infectious progeny in Vero cells	Inhibits viral replication by preventing viral adsorption, entry and replication.	(Albulescu et al., 2017; Tan et al., 2017)
ZINC33683341 and ZINC49605556	*In silico/In vitro*	Inhibits viral replication in Vero cells	Inhibits the function of protein E by binding the viral receptors	(Sandun et al., 2016)
25-hydroxycholesterol	*In vitro/In vivo*	Inhibits viral infection *in vitro* (A549, BHK-21, HeLa and Vero cells), reduces the morbidity and mortality in mice and considerably reduces fever and viremia in monkeys	Inhibits viral entry possibly blocking viral internalization	(Li et al., 2017a)

7. Conclusions

Current studies suggest that ZIKV produces an ensemble of structurally different virions circulating in an organism, collectively contributing to virus dissemination and tissue tropism. Considering that apoptotic mimicry may be used by ZIKV as an entry mechanism and that prM has also showed to be able to mediate this process, the release of immature particles may be advantageous for the virus, as it would increase the range of ZIKV susceptible cells. Thus, the degree of viral particle maturation and the amount of prM, E and PS exposed on the surface of the virus seem to be determinant in ZIKV entry mechanism in cells and appear to safeguard viral evolutionary processes of infectivity. Further studies regarding the role of viral particle maturation, the tropism and the cellular receptors involved in ZIKV entry are needed.

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Declarations of interest

None.

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