JSIANE : Vol. 3, Issue 3, 2023, Page 20–32 ISSN (Online) 2583-2093

Journal of Science Innovations and Nature of Earth

Journal homepage : www.jsiane.com

A REVIEW ON THE RECEPTORS AND PATHWAYS USED BY THE DENGUE VIRUS TO INFECT HOST CELLS

Anil Kumar[1], Hridayesh Arya[2], KalpanaSoni[3], Prveen[4] , Vishan Kumar[5], Ajay Pratap Singh[6], Surbhi Mittal[7], Manish Maheshwari[8], Prem Sagar[9], Anand Pratap Singh[10], Sonal Singh[11] and Keshav Singh[12]

[1,2&5] Department of Zoology, N.R.E.C. College, Khurja (Bulandshahr), Affiliated to C.C.S. University, Meerut, UP. India.

[3]Department of Zoology, Govt. Raj Rishi (Autonomous) College, Alwar - 301001, Rajasthan

[4]District VBD Specialist, IMEP-2, NVBDCP, Ministry of Health and Family Welfare, Government of India

[6]Department of Zoology, Dr. Bhimrao Ambedkar Government College, Odanya Padariya, Mainpuri, Affiliated to Dr. Bhimrao Ambedkar University, Agra, Uttar Pradesh, India

[7]Department of Zoology, Kisan (PG) College, Simbhouli, Hapur, Uttar Pradesh, India

[8]Department of Zoology, DS College, Aligarh, Uttar Pradesh, India

[9]Department of Zoology, Govt. (PG) College, Jalesar, Uttar Pradesh, India

[10,11,&12] Department of Zoology, Agra College, Agra, Affiliated to Dr. Bhimrao Ambedkar University, Agra, Uttar Pradesh, India

Email: anilkashyapnrec@gmail.com

www.doi.org/10.59436/jsiane.com/archives3/3/96

Abstract

Dengue fever is caused by a virus that belongs to the Flaviviridae family. Around 400 million individuals worldwide contract the mosquito-borne disease dengue each year, which has a 20% fatality rate in patients who have the most severe cases. People may contract many acute DENV infections during their lifetimes because there are four DENV serotypes, each of which has some immunologic cross-reactivity. A vaccine that is active against all four DENV serotypes has not yet been created. Studies on the virus's receptors and transmission pathways identified putative host targets crucial for virus internalization and propagation in host cells. In this review article, we'll cover information about potential receptors and attachment sites in mammalian and mosquito cells. We'll also go through the many entry points and mechanisms that enable the viral genome to reach the cytoplasm and lead to the internalization of the virus.

***Keywords*:** non-structural proteins, structural proteins, Dengue virus, entry routes, receptors.

Received 13.04.2023 **Revised 25.07.2023** **Accepted 23.09.2023**

Introduction

An estimated 96 million people are afflicted with this endemic arboviral disease (Mutheneni *et al*., 2017; Bhatt *et al*., 2013), and symptoms vary in intensity across affected populations. Dengue fever has been on the rise worldwide for decades, with millions of afflicted people putting a financial strain on Southeast Asia and the Indian subcontinent (Hussain *et al*., 2015). The *Aedes* species is the primary vector of the Flavivirus infection that causes dengue, as stated by Kumar *et al*. (2023), Mutheneni *et al*. (2017), and Hawley *et al*. (1987). Four serotypes of DENV (1–4) have been identified in several parts of the world, as reported by Holmas (1998). Antigenically speaking, these four serotypes of the dengue virus are distinct from one another. In 2013, Malaysian researchers identified the fifth dengue virus serotype (DENV-5) through isolation and genetic sequence analysis (Mustafa *et al*., 2015).

Dengue fever has been more common in the previous two decades, and researchers have begun to notice a shift in the disease's pattern of regional spread. The dengue virus's molecular structure, infecting mechanism, and life cycle have all been revealed by several studies. The dengue virus uses a variety of receptors and other cell surface components to infect new hosts during its life cycle (Table 1). Virus particles are internalized with the aid of cell surface components. Viruses are taken in by the host cell via receptor-mediated endocytosis once the envelope protein makes contact with a surface molecule or receptor. The virus RNA can enter the host cell cytoplasm because the virus envelope protein merges with the endosomal membrane during internalization. The single-stranded, positive-sense RNA that makes up the dengue virus's genome is translated into both structural and non-structural proteins by the host cell's machinery. Dengue virus replication occurs in host cells, where new RNA copies are made and the viral genome is copied. The Endoplasmic reticulum and Golgi apparatus play important roles in the assembly and development of the Dengue virus. The trans-Golgi network is responsible for the secretion of the mature viral particle (Mukhopadhyay, 2005).

Information on possible receptors and attachment sites in mammalian and mosquito cells will be discussed. We've also covered the many mechanisms by which the viral

genome can get access to the cytoplasm and trigger internalization.

Organization of DENV particle

Using the host's translation machinery, the dengue virus's 11 kilobases (kb) genome encodes 3 structural and 7 non-structural proteins. Structural proteins of molecular weights of 11 kDa, 21 kDa, and 53 kDa are encoded by the viral genome. These proteins include the capsid, pre-membrane, and envelop proteins. NS-1, NS-2A, NS2-B, NS-3, NS-4A, NS4B, and NS-5 are seven non-structural proteins that are encoded by the viral genome and have various biological functions (Bäck and Lundkvist, 2013; Kumar *et al.,* 2023). Capsid proteins construct the nucleocapsid core that encloses viral RNA. Host cell lipids enclose the nucleocapsid, and many Envelop (E) and Pre-membrane (prM) proteins with their respective I, II, and III domains are linked together (Rodenhuis-Zybert *et al*., 2010). To combat an infection with the dengue virus, the host immune system will seek out and attack the virus's prM and E proteins (Whitehead *et al*., 2007). The 3' untranslated region (3'UTR) of the dengue virus genome contains many conserved RNA sequences that are essential for virus replication. The 5'-untranslated region (UTR) is responsible for regulating the stability, translocation, and translation efficiency of viral mRNA (Ng *et al.,* 2017). Non-structural proteins play an important part in viral replication and maturation, although these proteins have distinct biological functions. Protease activity is provided by both NS2B and NS3, while NS3 also functions as a helicase, serine protease, nucleotide triphosphate, and 5'RNA triphosphatase. NS5 is an RNA-dependent RNA polymerase (RdRp) and methyltransferase (MTase). According to several studies (Zou *et al.,* 2011; Apte-Sengupta *et al.,* 2014; Luo *et al.,* 2015; Obi *et al.,* 2021), NS2B-NS3, NS4B, and NS5 are the primary targets for the development of antiviral medicines and vaccines.

Transmission routes of dengue virus

Two different environments serve as conduits for the spread of DENV: cities and forests. Both cycles are transmitted to hosts, such as monkeys, by the *Aedes aegypti* and *Aedes albopictus* mosquitoes. When female mosquitoes feed on blood in metropolitan settings, DENV enters the midgut and, after 8 to 12 days, spreads to the salivary gland and buccal cavity, where it finally infects people. In a similar vein, non-human primates found in the forest serve as natural hosts for DENV infection. The two cycles can interact significantly, hampering the prevention of illness, as the line between cities and the wild gets closer.

Interaction of virus to host cell surface

The dengue virus is spread by female *Aedes aegypti* mosquitoes that have been infected. The plasma membrane receptor of the host cell is a target for the dengue virus envelop protein. Viruses can be recognized in part because of chemicals or receptors in membranes (Table 2). Target cell receptors are ideal targets for the development of antiviral medications because their position and abundance play a key influence in the host tissue's susceptibility to the virus. The initial interaction between the virus and the host cell is usually triggered by attachment factors present on the cell surface. After a non-specific interaction has delivered the virus to the cell surface, it is more likely to bind to its specific receptor, a molecule that serves as the door for virus entry into the target cell (Grove and Marsh, 2011). Despite efforts to identify the molecules responsible for target cells' identification of DENV, no DENV receptor has been found (Hidari and Suzuki, 2011). Dendritic cell adhesion molecules (DC-SIGN), macrophage mannose receptor (MR), CD4 lipopolysaccharide receptors (LPS), heat shock proteins (HSP70/90), and chaperonin GRP78 are good options of diverse nature in mammalian (Table 1) and mosquito cells (Table 2).

Dengue virus has different serotypes that targets different cell in mammals and mosquito, that are vulnerable to infection. Dengue virus binds to various molecules or receptors in a serotype-specific manner, not using only a single specific receptor for internalization into host cells. Additionally, the virus can infect a range of cell types that are highly unlike one another while within these hosts. Therefore, the fact that DENV can connect to and penetrate host cells through a wide variety of molecules is not surprising. In fact, the search for the DENV receptor raises the possibility that the virus evolved to be non-specific by expanding the range of its targets rather than having a single target cell or organ. This represents an extensive viral dissemination throughout the host organism in vivo, which results in the vast spectrum of clinical symptoms observed in DENV-infected patients.

Based on the available data, the envelope (E) protein's domain III contains the receptor binding site. In the mammalian cell lines BHK-21 and HepG2, as well as the *Aedes albopictus* cell line C6/36, the recombinant envelope protein domain-III inhibits dengue virus entry (Hung *et al.,* 2004; Chin, 2007). The dengue virus cannot bind to Vero cells when antibodies against domain-III of the enveloped protein are present (Crill and Roehrig, 2001). However, because the cellular receptors for DENV are unknown, it is difficult to determine specific binding motifs within protein E.

GAGs (glycosaminoglycans) are probably involved in DENV attachment. Following the incubation of the virus, DENV infection was reduced by heparin, highly sulfated heparan sulfate, or suramin (Chen *et al.,* 1997; Lee *et al.,* 2006; Artpradit *et al.,* 2013). DENV infection was also prevented by pre-treating cells with heparin, heparan sulfate, or heparinase III. Two lysine residues in E protein DIII (K291 and K295; see Fig. 1) have been demonstrated to be critical for DENV interaction with GAGs through site-directed mutagenesis studies (Watterson, 2012).

In agreement, recombinant DIII binding to BHK-21 cells was totally eliminated by heparin treatment. Additionally, DIII exhibits the heparan sulfate-interacting motifs since it poorly binds to mutant cells that lack heparan sulfate (Hung *et al.,* 2004). The neutral glycosphingolipids' carbohydrate moieties are another putative DENV attachment factor in addition to GAGs. As a dengue virus-2 ligand, the glycosphingolipids are identified in AP-61 cells of the mosquito *Aedes pseudoscutellaris*. Dengue virus-2 recognizes the nLc4Cer (Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1rCer) in human erythroleukemia cell lines (K562) and rhesus monkey kidney cells (LLC-MK2) (Aoki *et al.,* 2006; Wichit *et al*., 2011).

Potential DENV receptors have been identified as dendritic cells (DC) (Wu *et al.,* 2000; Marovich *et al.,* 2001). One of the main objectives of DENV replication in vivo is DC. It was discovered that there was a positive correlation between

the expression of DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) or its homolog L-SIGN and DENV infection in endothelial cells from the liver and lymph nodes (Navarro-Sanchez *et al*., 2003; Tassaneetrithep *et al.,* 2003). DC infection by DENV was blocked by anti-DC-SIGN antibodies and overexpression of DC-SIGN in cells that don't normally make it made them susceptible to infection. Further evidence that this lectin has a role in the pathophysiology of dengue comes from human genetic studies, it linked the development of dengue hemorrhagic fever rather than typical dengue to a variation in the DC-SIGN promoter (Sakuntabhai *et al.*, 2005). The mannose-rich N-glycan on amino acid residue N67 of the E protein interacts with the DC-SIGN carbohydrate recognition domain (CRD) to bind DENV to DC-SIGN (Pokidysheva *et al.*, 2006).

It stands to reason that a virus's ability to infect DC will be influenced by its species of origin, given that insect and mammalian cells undergo distinct glycosylation processes. Viruses created in either the human host or the mosquito vector have a glycan structure that is rich in mannose and are therefore able to infect DCs (Hacker, 2009). N-linked complex glycans but not mannose-rich glycosylation are abundant in DENV isolated from DC (Dejnirattisai *et al*., 2011), which is an intriguing finding. These viruses inhibit DC infection by lowering DC's receptivity to DC-SIGN. However, viruses created in other cell types were able to infect both DC-SIGN and L-SIGN-expressing cells, but viruses obtained from DCs could only bind to cells expressing L-SIGN. The virus's infectivity and tropism, in other words, would be determined by the cell in which it assembles.

It was formerly believed that the main locations of DENV replication were monocytes and macrophages. It is more likely that virus entry through the Fc receptor or cell activation will be required for productive infection of these cells when non-neutralizing antibodies are present. The term "antibody-dependent enhancement" (ADE) describes these phenomena. However, the LPS receptor CD14 was the first candidate receptor for DENV binding to monocytes/macrophages. Researchers found that inhibiting DENV multiplication in cultured cells by introducing LPS prior to infection did so without eliciting an inflammatory response.

Because DENV binding to monocytes and macrophages depends on a cell surface protein that is part of the LPS-CD14 complex, blocking CD14 likely completely antagonizes the inhibitory effects of LPS on DENV infection (Chen, 1999). Treatment with CD14 antibodies did not inhibit viral replication. Once it was discovered that heat-shock proteins HSP90 and HSP70 acted as ligands for E proteins or as complete DENV particles, a new explanation for these findings became available. Since it was found that both proteins are LPS receptors (Chen *et al.*, 1999; Reyes-Del Valle *et al.*, 2005), HSP90 and HSP70 may be the molecules that are rendered inactive for DENV binding upon initial cell exposure to LPS. Incubation of primary human monocytes and macrophages with antibodies against heat shock proteins 90 and 70 inhibited DENV replication.

It has been suggested in recent years that the mannose receptor (MR) functions as a DENV receptor in macrophages. Anti-MR antibodies inhibited DENV infection when incubated with macrophages (Miller *et al.*, 2008), whereas the surface MR expression of 3T3 cells, a cell line derived from mouse embryos, promoted viral attachment. Evidence for binding via the MR was also provided by the authors. Given that CRD can identify N-linked glycans in the E protein's amino acid residues N67 and/or N153, it is conceivable that CRD can identify the recombinant E protein and all four DENV serotypes, but not its deglycosylated counterpart.

It has been discovered that hepatocytes may have the ER resident chaperonin GRP78 as a DENV receptor. Although GRP78 is generally found inside the cell, it has been shown to localize to the cell membrane. After incubation with antibodies against GRP78, Jindadam (2004) and Upanan (2008) found that DENV-2 was unable to infect the hepatic cell lineage HepG2. These scientists also looked into how DENV-1 interacted with HepG2 cells. They discovered that, unlike DENV-2, DENV-1 attaches to and enters liver cells via the high-affinity laminin receptor. The fact that soluble laminin or antibodies against the laminin receptor did not affect dengue virus types 2, 3, or 4 infections showed that this interaction was unique to DENV-1 (Thepparit and Smith, 2004). In a recent study using many cell types, including human embryonic stem cells, it was found that transmembrane receptors belonging to the TIM and TAM families have a role in DENV infection of mammalian hosts.

Phagocytic engulfment and clearance of apoptotic cells is a function of these proteins, and they can't do their job without phosphatidylserine (PS). DENV interaction with TIM and TAM molecules appeared to improve virus endocytosis and was reliant on viral envelope PS, as reported by Meertens *et al*. (2012). Antibodies targeting TIM and TAM, as well as their knockdown via RNA interference, protected mice from DENV infection. According to the data acquired by Meertens and coworkers (Perera-Lecoin *et al.,* 2014), apoptotic clearance may be exploited to facilitate virus entry and infection.

Remember that the prM protein directly interacts with claudin-1, the main structural element of tight junctions. Claudin-1 expression is increased early in a DENV infection, which may facilitate virus entry into host cells (Gao *et al.*, 2010; Che, 2013). One co-receptor for HCV entry, for instance, has previously been identified as claudin-1 (Meertens *et al.*, 2008; Liu *et al.*, 2009; Harris *et al.*, 2010). Some viruses gain entry to host cells using tight junction proteins. Some studies suggest that claudin-1 functions as a receptor for DENV entry by binding to DENV particles that are either immature or partially mature but still contain the prM protein. As the infection progresses, claudin-1 expression diminishes, which is interesting since it reduces the possibility of a super-infection by blocking the entry of a second wave of incoming virus particles.

Multiple studies have used the experimental viral overlay protein-binding assay (VOPBA) to identify new compounds that bind to DENV in distinct mammalian cell types. Numerous DENV-binding compounds have been identified through this research. For example, a 65 kDa protein that binds DENV-2 was found by Ramos-Castaneda *et al*. (1997) in the membranes of Vero cells, mouse and human neuroblastoma cells (N1E-115 and SK-NSH, respectively). In Vero cells, it has been demonstrated that these proteins (44 and 74 kDa, respectively) bind DENV-4. In HepG2

membrane preparations, distinct proteins have been found to bind each of the four DENV serotypes (Jindadamrongwech and Smith, 2004), and the endothelial cell line ECV304 (Wei *et al.*, 2003). As far as we are aware, no more characterization studies of these compounds have been conducted.

Table 1 : Putative receptors for dengue virus in human cells.

Cells	Surface receptor	Types	Serotype	References
U937 SK-SY-5Y Monocyte-derived macrophage	HSP90 HSP70	Protein	DENV2	Chen *et al*. (1990), Reyes-Del Valle *et al*. (2005)
Vero CHO	Heparin sulfate	GAG	DENV 2	Chen *et al*. (1997)
N1E-115 SK- NSH	65 kDa	Protein	DENV2	Ramos-Castaneda *et al*. (1997)
Monocyte/ macrophage	CD4	Protein	DENV2	Chen *et al*. (1999)
Vero	44, and 74 kDa	Protein	DENV4	Martinez-Barragan and del Angel (2001)
ECV304	29, 34 and 43 kDa	Unknown	DENV2	Wei *et al*. (2003)
THP-1 Monocyte-derived dendritic cells	DC-SING	C-type lectin	DENV2	Navarro-Sacnchez *et al*. (2003), Tassaneetrithep *et al*. (2003)
HepG2	GRP78	Protein	DENV2	Jindadamrongweh *et al*. (2004), Upanan *et al*. (2008)
HepG2	Diverse	Protein	DENV2, 3 and 4	Jindadamrongwech and Smith (2004)
HepG2 PS clone D	High-affinity laminin	Protein	DENV1,2 and 3	Thepparit and Smith (2004)
K562 BHK-21	nLc4cCer (Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1'Cer)	Glycosphingolipids	DENV2	Aoki *et al*. (2006), Wichit *et al*. (2011).
NIH3T3 Monocytes/ macrophages	Mannose receptor	Protein	DENV 1, 2, 3 and 4	Miller *et al*. (2008)
Huh-7 Huh-7.5	Claudin -1	Protein	DENV2	Gao *et al*. (2010), Che *et al*. (2013)
A549 Huh7 5.1 Vero	TIM-1	Protein	DENV2, and 3	Meertens *et al*. (2012)
A549 Vero Human primary kidney epithelial cells and astrocytes	AXL	Protein	DENV 2 or DENV 3	Meertens *et al*. (2012)

Table 2 : Putative receptors for dengue virus in insect cells.

Cells/ Tissue	Surface Receptor	Type	Serotype	References
C6/36 cells Diverse mosquito tissues	40 and 45 kDa	Glycoprotein	DENV 4	Salas-Benito and del Angel (1997) Yazi Mendoza *et al*. (2002) Rayes-del Valle and del Angel (2004)
C6/36 cells	50 kDa	Protein	DENV 2, 3 and 4	Sakoonwatanyoo *et al*., (2006)
***Aedes aegypti* salivary glands**	77, 58, 54 and 37 kDa	Unknown	DENV 1, 2, 3 and 4	Cao-Lormeau (2009)
***Aedes polynesiensis* salivary glands**	67, 56, 54, 50 and 48 kDa	Unknown	DENV 1 and 4	Cao-Lormeau (2009)
C6/36 CCL-125	Prohibitin	Protein	DENV 2	Kuadkitkan *et al*. (2010)
C6/36 *Aedes aegypti* midgut	67 (enalase) and 80 kDa	Protein	DENV 1, 2, 3 and 4	Mercado-Curiel *et al*. (2006); Mercado-Curiel *et al*. (2008); Munoz Mde *et al*. (2013)

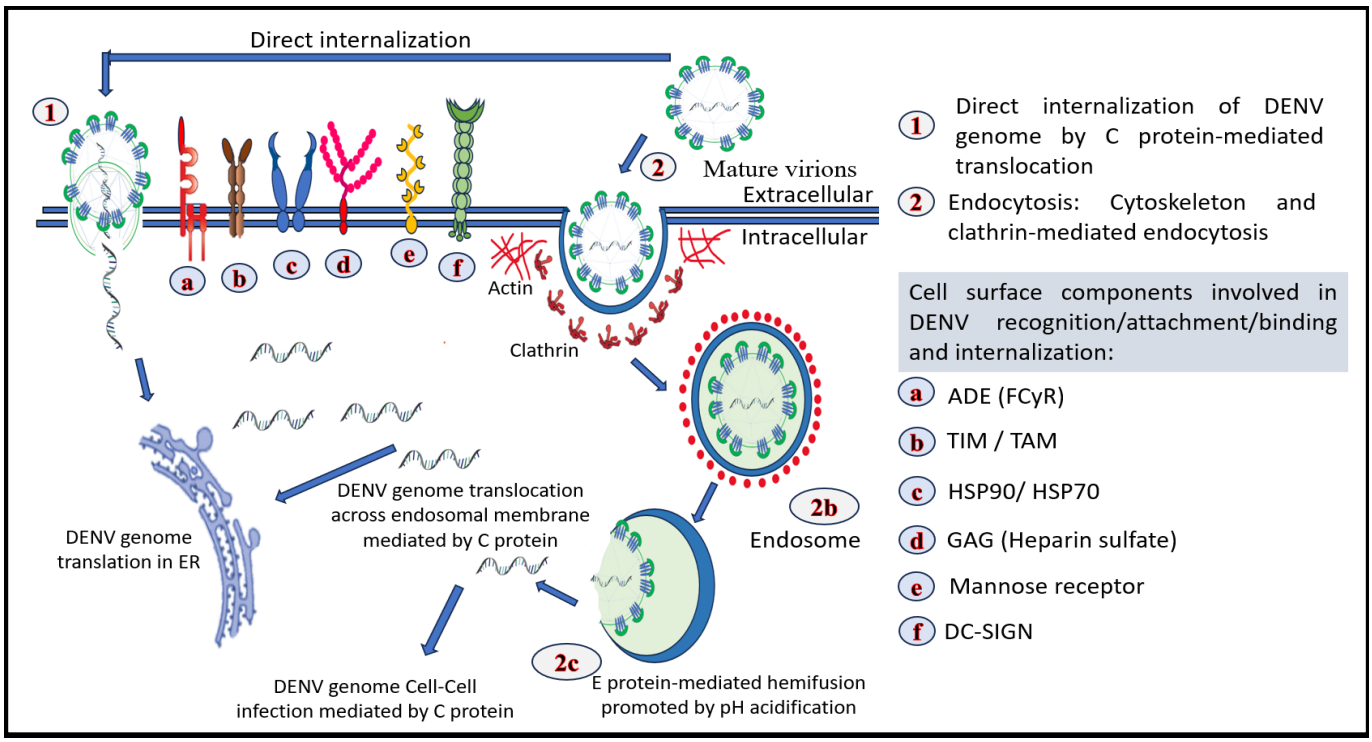

Fig. 1 : Entry routes of DENV into host cell. The first time DENV interacts with host cells, it binds to receptors or attachment factors on the cell surface. Upon recognition, direct cell membrane transfer may allow the virus genome to enter the cytoplasm. which would be carried out by receptor-mediated endocytosis or by C protein-dependent membrane translocation. Cellular elements for endocytic pathways, such as the cytoskeleton and clathrin, are being recruited (2). The endosomal medium's pH is lowered (2b), which causes structural changes in the E protein (homodimer to trimer). E protein trimer-mediated lipid mixing and hemifusion is favored by changes in the endosomes' lipid composition (2c). As a result of either pore formation or C protein membrane translocation, the DENV genome is released into the cytosol (inset). The DENV genome moves from the cytosol to the ER for translation and replication. As an alternate method of virus dissemination in the host, the C protein's translocation characteristics allowed the viral genome to be transported to the neighboring cells (inset).

Prohibitin protein is the potential DENV receptor for the mosquito-borne DENV vector. C6/36 cells, a cell line generated from the larval stage of *A. albopictus*, CCL-125 cells, a cell line derived from *A. aegypti*, and adult *A. aegypti* mosquitoes were used in VOPBA and mass spectroscopy investigations to identify this protein as a DENV-2 ligand (Kuadkitkan *et al.*, 2010). Both cellular anti-prohibition antibody treatment and mRNA silencing were effective in inhibiting DENV replication. Supporting its role as a DENV receptor, studies using immunoprecipitation and colocalization imaging demonstrated that prohibitin selectively interacts with the DENV E protein (Kuadkitkan *et al.*, 2010).

Two surface-expressed glycoproteins have been reported to be expressed by C6/36 cells. one of 40 kDa and the other of 45 kDa, both of which have been shown to bind DENV-4 (Salas-Benito and Angel 1997). Salas-Benito and Angel (1997) reported the discovery of DENV-4. Antibodies against these molecules block the virus from adhering to the cells, bolstering the receptor function of the cells. Both proteins were detected in the midgut, ovary, and salivary glands of *Aedes aegypti* throughout their life cycles (Yazi Mendoza *et al.*, 2002), They are all recognized to be DENV infection-friendly mosquito organs. An additional method used to show the distinct binding of gp45 to the recombinant E protein was affinity chromatography (Reyes-del Valle & del Angel, 2004). In the end, gp45 was successfully detected by antibodies against HSP90, which has been proposed to play a part in the interaction between DENV and mammalian cells (Chen *et al.*, 1999; Reyes-Del Valle *et al.*, 2005). This implies that gp45 might function as an attachment factor as opposed to an entrance receptor since, following heat shock, it migrates to the cell surface and is associated with an increase in virus binding but not with viral replication.

A 50 kDa DENV-binding protein that was also identified by VOPBA was shown to be detected by the antibody against human high-affinity laminin, which was previously believed to represent the DENV-1 receptor in hepatic cells (Thepparit *et al.*, 2004). DENV serotypes 3 and 4 could not be replicated in mosquito cells treated with anti-laminin receptor antibodies or soluble laminin; however, DENV serotypes 1 and 2 were not suppressed, indicating that mosquito laminin receptors are not sensitive to DENV serotypes 1 and 2. Salas-Benito and Angel (1997) suggested that the gp45 protein that was previously found might be the same protein as the laminin receptor.

It has been discovered that two more proteins, measuring 67 and 80 kDa, respectively, bind to each of the four DENV serotypes (Munoz *et al.*, 1998). These proteins have been given the designations R67 and R80. These compounds were isolated from *Aedes*

aegypti midguts and C6/36 cells using affinity chromatography. Inhibition of dengue virus attachment and infection of C6/36 cells by antibodies against these molecules suggests they may function as dengue virus receptors in mosquitoes (Mercado-Curiel *et al.,* 2006). DENV infection susceptibility in mosquito strains has been traced back to R67 since 2008 (Mercado-Curiel, 2008). It was found to be associated with DENV infection and vector competence depending on where in the mosquito's midgut it was found. Enolase was identified as a 67 kDa protein, or the R67 protein, by proteomic analysis of *A. aegypti* midgut extracts that had been exposed to affinity chromatography with the recombinant E protein or DENV particles (Munoz Mde *et al.,* 2013).

Dengue patients have increased plasma levels of this protein because DENV infection triggers hepatic cells to generate more enolase (Higa *et al.,* 2014). It is reasonable to postulate that the hemostatic dysfunction seen in dengue patients, such as activation of fibrinolysis and changes in vascular permeability, is related to the increased synthesis of -enolase by infected hepatic cells. The reason for this is that by attaching to plasminogen, -enolase modulates its activation. The ability of enolase to bind to DENV in mosquito cells has not been tested in humans.

DENV is transmitted to humans by mosquito bites, with the virus entering the body via the vector insect's saliva. Due to the high interest in locating the virus receptor in mosquito salivary glands, we now know that DENV replicates quite well there. In addition, Cao-Lormeau (2009) reported that VOPBA was utilized to detect the DENV receptor in mosquito salivary glands. Five SGE proteins from *A. polynesiensis* were shown to bind to dengue virus types 1 and 4 in this study. The proteins' actual composition, however, is still a mystery. *A. aegypti* salivary gland extracts (SGE) contained four proteins with molecular weights of 77, 58, 54, and 37 kDa that bound to the four DENV serotypes.

Virus Internalization

Based on the virus strain and cell host, DENV can be endocytosed using either clathrin-mediated or non-classical methods (Table 3 summarizes these processes). Additional barriers to virus entrance include virus maturation and Fc receptor recognition of viral immunocomplexes.

DENV entry pathways

DENV-2 is taken up by most cell types via clathrin-dependent endocytosis (Table 3), including C6/36, HeLa, A549, Huh7, HepG2, and BS-C-1 cells. Acosta *et al*. (2009) report that the Vero cell entrance process is dynamin-dependent but not clathrin-dependent, caveolae-dependent, or lipid raft-dependent.

Acosta *et al*. (2008) and Mosso *et al*. (2008) report that clathrin-dependent endocytosis is used by all four DENV serotypes to enter C6/36 cells. Acosta *et al*. (2008) found that actin filaments, rather than microtubules, are involved in DENV-2 cell trafficking, indicating that the virus may not need to go from early to late endosomes in order to infect a host. Krishnan *et al*. (2007) observed that the virus fusion profile in early endosomes was likewise comparable in HeLa cells. However, the bulk of DENV particles are moved from early endosomes to late endosomes, where the virus stays for around five minutes before membrane fusion, according to a single-particle tracking study (Van der Schaar *et al.,* 2008; Smit *et al.,* 2011). Even so, green monkey kidney BS-C-1 cells also ingest DENV-2 by clathrin-mediated endocytosis.

The serotype of DENV seems to affect the entry point. In contrast to DENV-2, which does not require clathrin for entrance into Vero cells (Acosta *et al.,* 2009), DENV-1 does so. Additionally, variations among serotype-identical viral strains were discovered. Virus fusion in DENV-2 16681 infection in the same cell type occurs in perinuclear recycling endosomes, as reported by Acosta (2012), whereas in DENV-2 NGC infection, virus transport to late endosomes is Rab7 dependent. Researchers have found that DENV-2 can be silenced in hepatic cells by utilizing small interfering RNA (siRNA), and that this pathway is used by all DENV serotypes to enter Huh7 cells. Silencing certain genes allowed researchers to demonstrate that clathrin-mediated endocytosis was required for DENV entry into HepG2 cells (Alhoot, Wang, and Sekaran, 2012). In addition to clathrin-mediated endocytosis, macropinocytosis was found to be one of the entrance routes for DENV in these cells (Suksanpaisan, 2009). According to Meertens *et al*. (2012), DENV entry through macropinocytosis is facilitated by TIM and TAM receptors' recognition of PS within the viral envelope. Parts of the viral membrane, including PS, are exposed by DENV's structure at 37°C, which is the normal temperature of mammalian cells (Meertens *et al.,* 2012; Fibriansah *et al.,* 2013; Zhang *et al.,* 2013a). It has been demonstrated that a number of other viruses may infect cells in this manner (Mercer and Helenius, 2008; Jemielity *et al.,* 2013). One of these viruses that exploits apoptotic mimicry is DENV, since PS exposure is a trigger for apoptosis.

It's interesting to note that different cellular mechanisms can use different DENV entry pathways to spread the virus. Unlike DENV, which utilizes a clathrin-mediated endocytic route to replicate serially in Vero cells, DENV that is created in C6/36 cells uses a non-classical clathrin-independent method of entry (Acosta *et al.,* 2014). Acosta *et al*. (2014) observed that a virus that was propagated serially in Vero cells had a decreased affinity for cellular heparan sulfate. This variation suggests that mutations have occurred in the E protein, leading to a more positive net charge (Lee *et al.,* 2006; Prestwood *et al.,* 2008; Anez *et al.,* 2009). However, it is yet unclear if this would explain why viral particles made in insect cells take a different endocytic route than those made in mammalian cells. Changes in the E protein glycosylation locations inhibited virus infectivity and spread in human cells but had no effect on virus growth in insect cells, suggesting that DENV E protein glycosylation is critical for virus tropism (Bryant *et al.,* 2007; Mondotte *et al.,* 2007).

Table 3: DENV entry pathways.

Target cell and Serotype	Cell used for virus propagation	Endocytic pathway	Endocytic trafficking	Reference(s)
C6/36 and DENV2	C6/36	Clathrin-dependent	Early endosomes Early to acid lysosomes	**Acosta *et al.* (2008)** **Mosso *et al.* (2008)**
Vero and DENV2	C6/36	Non-classical dynamin-dependent (Clathrin, caveolae and lipid raft-independent)	Early to late endosomes (DENV 2 strain 16681) Early endosomes to recycling endosomes (DENV 2 strain NGC)	**Acosta *et al.* (2009, 2012, 2014)**
Vero and DENV2	Vero	Clathrin-dependent	N.D.	
Vero and DENV 1	C6/36		Early endosomes to recycling endosomes (DENV 1 strain HW)	
HeLa and DENV2	C6/36	Clathrin-dependent	Early endosomes (strain NGC) Early to late endosomes (strain PR159 S1)	**Krishnan *et al.* (2007)and Van der Schaar *et al.* (2008)**
BS-C-1 and DENV2	C6/36	Clathrin-dependent	Early to late endosomes	**Van der Schaar *et al.* (2008)**
A549 and DENV2	C6/36	Clathrin-dependent	N.D.	**Acosta *et al.* (2009)**
Huh 7 and DENV2	C6/36	Clathrin-dependent	Early to late endosomes	**Ang *et al.* (2010)**
HepG2 and DENV2	C6/36 or Vero	Clathrin-dependent	N.D.	**Alhoot *et al.* (2012) A**
HepG2 and DENV1, 2, 3 and 4	Vero	Micropinocytosis	N.D.	**Suksanpaisan *et al.* (2009)**
HepG2 and DENV1, 3 and 4	Vero	Clathrin dependent	N.D.	

N.D.: Not determined.

As previously mentioned, the prM/M protein in DENV immature particles regulates changes in E protein shape that are crucial for maturation and infection. The pr peptide is still linked to the E protein even after prM is broken down by furin, avoiding conformational alterations that may trigger its fusogenic action in TGN. Importantly, furin is a TGN protease that is also found on cell surfaces and in early endosomes which may help to explain why DENV immature particles are so infectious (Shapiro *et al.*, 1997; Zybert *et al.*, 2008).

It is possible that immature DENV particles may bind to and enter the body if anti-prM antibodies were present. PrM would then be cleaved by furin in endosomes to allow for viral maturation and E protein-mediated membrane fusion (Rodenhuis-Zybert *et al.*, 2010). The acidic pH (5.0) of endosomes would also aid the viral particle's loss of pr, allowing the virus a greater ability to produce fusion proteins (Zheng *et al.*, 2010). Interestingly, the pr subunit of prM has been shown to interact with vacuolar ATPase (Duan *et al.*, 2008), however it is still unclear whether or not this link would impact the acidity of the endosomal media when immature viruses enter. Despite reported differences between cell types, DENV infection usually results in the production of immature particles or particles with varying degrees of maturation (van der Schaar *et al.*, 2007; Zybert *et al.*, 2008; Junjhon *et al.*, 2010; Rodenhuis-Zybert, Wilschut, and Smit, 2011). Completely mature DENV particles can infect without the prM endosomal furin-processing activity or the binding of anti-prM antibodies, whereas completely immature DENV particles require both of these for infection. However, for DENV particles that are not fully mature and still include prM, it is unclear the function of antibodies, internalization via FcR, and furin cleavage.

In these situations, increase of viral binding by antibody-mediated furin processing of prM may or may not be required for infection. as reported by Rodenhuis-Zybert *et al.* (2011). Given that the production of immature and partially immature viruses is an integral aspect of DENV's life cycle, prM antibodies, and furin activity in early endosomes are anticipated to have a major impact on the virus's ability to infect and cause disease.

Genome access to cytoplasm

The fusing of the viral envelope with the cellular membrane is mediated by a viral fusion protein that is anchored at the virion envelope. This process permits the viral genome to enter the cytoplasm. Although DENV can enter cells through several different entry points. According to Peterson and Kielian (2013), the current notion is that the

virus's genetic material enters the cytoplasm via membrane fusion mediated by E proteins. However, according to recent research, the C protein may also play a role in the diffusion of DENV RNA into the cytoplasm (Freire *et al.*, 2013a, b; Freire *et al.*, 2014).

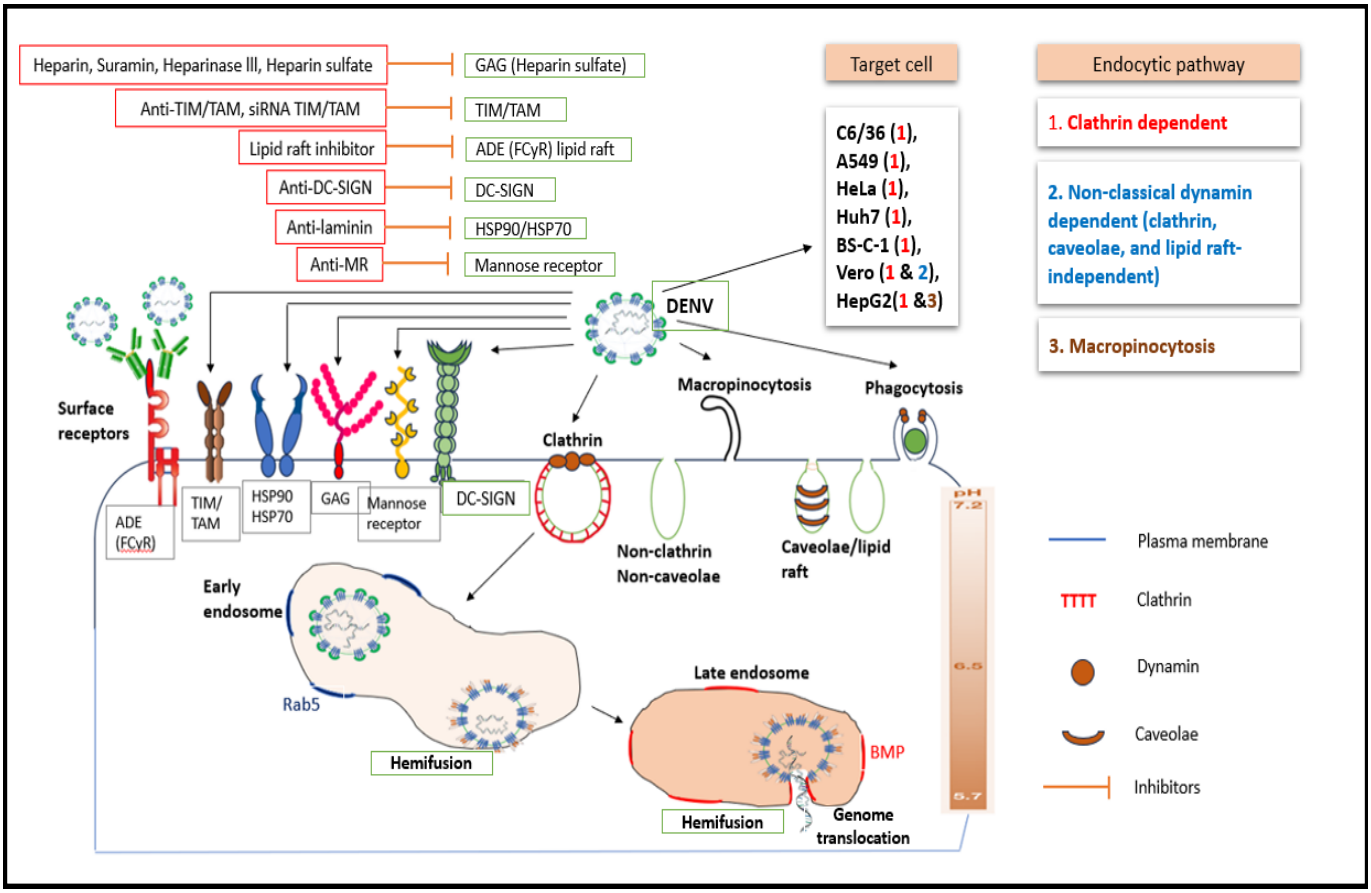

Fig. 2 : The several ways in which DENV enters its host cell. Several cell surface receptors have been identified by the use of diverse experimental strategies, including receptor analogues as inhibitors, siRNA, antibodies, and the inhibition of lipid raft production. DENV can enter the host cell by clathrin-mediated endocytosis, micropinocytosis, or non-classical dynamin-dependent (clathrin, caveolae, and lipid raft independent) endocytosis, among other methods, depending on the host cell and the DENV serotype. The endosomal medium has an acidic pH during endocytic trafficking. Hemifusion between the endosomal membrane and the viral membrane is facilitated by alterations in the E protein confirmed by the acidic pH of the endosome. The timing of viral membrane fusion and endosome formation is also dependent on the type of cell being infected. The maturation of DENV requires the TGN protease furin, which is found in the early endosome, and the BMP (a particular anionic lipid) of the late endosome, where the structural protein prM of the immature virus is cleaved. Depolymerization of microtubules has been hypothesized to prevent fusion or genome translocation into the host cytoplasm. Viruses employ intraluminal vesicles for transport between early and late endosomes.

E protein-mediated membrane fusion

Different forms of viral fusion proteins can be distinguished by their structure (Da Poian, 2005; Harrison, 2008; Modis, 2014). According to Peterson and Kielian (2013), class II fusion proteins are hydrophobic fusion loop-covered in the dimer interface, beta-sheet-rich proteins that produce homodimers at neutral pH. These proteins are present in alphaviruses and flaviviruses. A class II second protein links them to the DENV-specific M protein, which is cleaved during viral assembly to form a pH-responsive structure (Li *et al.*, 2008).

In recent years, a detailed description of the DENV fusion mechanism has been made possible by several structural studies on E protein reorganization at different pH (Modis *et al.*, 2003, 2004), temperatures (Fibriansah *et al.*, 2013; Zhang *et al.*, 2013a), and in the pre-fusion and hemifusion states (Klein, 2013). In order for the DENV fusion process to occur at the endosomes, low pH promotes numerous structural changes in the E protein (Modis *et al.*, 2004). Before the fusion loop can be incorporated into the outer leaflet of the endosomal membrane, the E protein homodimer dissociates and reorganizes into trimers (Modis *et al.*, 2004; Zhang *et al.*, 2004). The E protein folds in on itself to form a trimer of hairpin-like structures when the fusion peptide is attached to it, which causes the trans-membrane region to shift in the direction of the endosomal membrane. This structure, according to Modis (2014) and Pierson and Kielian (2013), guides the host and viral membranes toward the hemifusion and post-fusion stages, where a pore is generated through which the viral DNA can escape. HIV-1 and influenza virus, both of which contain class I fusion proteins, have been studied in great detail (Harrison, 2008; Melikyan, 2014), but the stages after

hemifusion for DENV or other flaviviruses have not yet been observed (Pierson and Kielian, 2013).

According to recent research, changes in the lipid composition of endosomal membranes along the endocytic pathway as well as pH-induced conformational changes of the DENV E protein may influence the molecular processes spanning virus internalization and viral genome release into the cytoplasm (Nour and Modis, 2014). Similar to plasma membranes, early endosome membranes benefit from sphingolipids and sterols, as stated by van Meer (2008). In the membrane of late endosomes, the anionic lipid bisphosphate (monoacylglycerol) (BMP) is synthesized, whereas the relative quantity of cholesterol and PS declines (Kobayashi *et al.,* 2002). According to Zaitseva *et al*. (2010), BMP inclusion in the late endosomal membrane is necessary for DENV membrane fusion (Fig. 2). As such, DENV can fuse with the insect cell's outer leaflet of the plasma membrane but not with the mammalian cell's plasma membrane (Nour and Modis, 2014). This is because the outside leaflet of insect cells' plasma membranes contains PS, an anionic phospholipid.

Single-particle tracking analyses of flaviviruses, however, showed that the process of endosomal maturation had little to do with the exchange of lipids between viral and cellular membranes. These investigations demonstrated that viral genome dispersion into the cytosol was several minutes ahead of lipid mixing. These findings imply that the viral entry pathway consists of two independent steps: membrane fusion and nucleocapsid translocation to the cytoplasm. More recent studies have questioned whether pH-induced E protein-mediated fusion is required for the DENV genome to get access to the cytoplasm (Nour *et al.,* 2013; Freire *et al.,* 2013b; Vancini *et al.,* 2013). Interesting findings included the idea that DENV might infect a host cell by breaking through its plasma membrane (Vancini *et al.,* 2013). Genome internalization does not necessitate endosome internalization or pH acidification, as tagged viral particles were identified at the plasma membrane with either the electron-dense genome or with no electron density at all (Vancini *et al.,* 2013). The viral protein responsible for this process was not specified. Recombinant DENV C protein may play this role since it transports functional nucleic acids into cells (Freire *et al.,* 2013b). This discovery has the potential to shed light on the process of direct cell entrance by DENV and provide new insight into the role of E protein-mediated fusion in the internalization of viral genomes.

RNA translocation through the membrane mediated by capsid proteins

In a recent article, a group of scientists called the capsid proteins of all flaviviruses "supercharged proteins" (Freire *et al.,* 2013b). According to Thompson (2012) and McNaughton *et al.* (2009), supercharged proteins are a family of proteins having a high charge/molecular weight (MW) ratio or net charge "density", that may rapidly enter cells and deliver functional payloads. Virus capsid proteins are thought to have evolved to have a high net charge/MW so that they could transport lipid membranes that contained viral nucleic acids into the cytosol. Nonenveloped viruses rely on their capsid proteins to connect to cells, and once attached, these proteins undergo conformational changes that allow viral DNA to cross the membrane (Suomalainen and Greber, 2013).

However, to our knowledge, the majority of study has focused on the membrane fusion process, with little attention paid to the role that encapsulated viruses' capsid proteins play in genome internalization. According to recent studies in this field, the DENV C protein, like all flavivirus capsid proteins, is thought to be involved in the internalization of the viral DNA. It was discovered that short oligonucleotide sequences (ssDNA and siRNA) could be translocated across cellular membranes by recombinant DENV C protein and two synthetic peptides that included C protein residues 45-72 and 67-100 (Freire *et al.,* 2013b, Freire *et al.,* 2014). Remarkably, large nucleic acids, such as a plasmid encoding GFP, were expressed at levels similar to those attained when lipofectamine or other common transfection agents were applied (Freire *et al.,* 2013b). They were also successfully delivered into the cells. Evidence indicates that because the DENV C protein may transmit functional nucleic acid molecules across cellular membranes, it may collaborate with E protein during the fusion stages (Fig. 1). We believe that during viral endocytosis, the trimeric form of the E protein binds to the endosomal membrane and encourages hemifusion of the viral and endosomal membranes. The DENV C protein facilitates the entry of viral RNA into the cytosol because of its ability to translocate across membranes. Recent studies of the Flavivirus genus, including those of Japanese encephalitis and yellow fever, corroborate this idea (Nour *et al*., 2013).

Virus particles may create metastable hemifusion intermediates with the tiny endosomal carrier vesicles (ECVs), as reported by Nour *et al.,* (2013). This takes place a few minutes prior to the components of the nucleocapsid being transferred into the cytoplasm. According to Zaitseva *et al*. (2010), this is consistent with the earlier description of "restricted hemifusion" intermediates during DENV entry. Different proteins, including E and C, may play roles in RNA translocation into the cytosol, the fusion process, and hemifusion, and these events may occur at different times.

Similar to how other viruses like HIV employ their C proteins to achieve cell-to-cell direct genome transfer (Hubner *et al.,* 2009; Zhong *et al.,* 2013), Nucleic acids can be transported across cellular membranes by DENV C proteins (Fig. 2). For viruses to spread in this way, they just need to be partially assembled and released from infected cells.

Concluding Remark

A virus's capacity to infect the host widely may be hampered if it uses a cell surface receptor that is found only in a subset of host cells or organs. DENV can infect a wide variety of cells in both its vertebrate hosts and the mosquitoes that feed on them. Rather than relying on a single, specialized receptor for its internalization, it is more likely that DENV recognizes and binds to a variety of molecules, presumably in a serotype or strain-specific manner.

At first, it was thought that most viruses only had one possible entry point, but as the field developed and high-resolution quantitative methods became accessible, researchers discovered and defined many entry points for many viruses. The spread of HIV is a classic case of this type of progress. HIV may enter the cell by endocytosis, despite its early limitations on class I membrane fusion and CD4 and CXCR4/CCR5 receptor binding, according to a number of lines of evidence (Daecke *et al.*, 2005; Miyauchi *et al.*, 2009; Padilla-Parra *et al.*, 2013; Melikyan 2014). According to Hubner *et al*. (2009) and Zhong *et al*. (2013), the main way that HIV spreads throughout the host is currently believed to be through cell-to-cell transmission.

Despite common knowledge, DENV can only replicate inside of endosomes. The structural changes in the E protein that occur in response to pH causes hemiffusion between the endosomal and viral membranes. The C protein may aid DENV RNA entrance into the cytoplasm by interacting with the E protein during the hemifusion state and serving as a viral genome translocator in the endosomes or at the cell membrane during direct cell infection. The finding that the C protein may transport nucleic acids across membranes provides support for the hypothesis that it may assist the direct cell-to-cell transmission of viral RNA.

Since this does not necessitate full particle creation and release from the infected cell, it represents a potential alternate route of viral transmission in the affected tissues. The C protein's ability to transport nucleic acids, like that of other capsid proteins, makes it a useful biotechnological tool for delivering siRNA and plasmids into cells (Usme-Ciro *et al.*, 2013).

Conflict of interest: No conflict of interest

References

Acosta, E.G., Castilla, V. and Damonte, E.B. (2009). Alternative infectious entry pathways for dengue virus serotypes into mammalian cells. *Cell Microbiol*., 11: 1533–49.

Acosta, E.G., Castilla, V. and Damonte, E.B. (2012). Differential requirements in endocytic trafficking for penetration of dengue virus. *PLoS One*, 7:e44835.

Acosta, E.G., Castilla, V., Damonte, E.B. (2008). Functional entry of dengue virus into *Aedes albopictus* mosquito cells is dependent on clathrin-mediated endocytosis. *J Gen Virol.*, 89: 474–84.

Acosta, E.G., Castilla, V. and Damonte, E.B. (2011). Infectious dengue-1 virus entry into mosquito C6/36 cells. *Virus Res.*,160:173–9.

Acosta, E.G., Piccini, L.E., Talarico, L.B. *et al*. (2014). Changes in antiviral susceptibility to entry inhibitors and endocytic uptake of dengue-2 virus serially passaged in Vero or C6/36 cells. *Virus Res*., 184: 39–43.

Alhoot, M.A., Wang, S.M. and Sekaran, S.D. (2012). RNA interference-mediated inhibition of dengue virus multiplication and entry in HepG2 cells. *PLoS One*, 7:e34060.

Anez, G., Men, R., Eckels, K.H. *et al*. (2009). Passage of dengue virus type 4 vaccine candidates in fetal rhesus lung cells selects heparin sensitive variants that result in loss of infectivity and immunogenicity in rhesus macaques. *J Virol*., 83: 10384–94.

Ang, F., Wong, A.P., Ng, M.M. *et al*. (2010). Small interference RNA profiling reveals the essential role of human membrane trafficking genes in mediating the infectious entry of dengue virus. *Virol J*., 7: 24.

Aoki, C., Hidari, K.I. and Itonori, S. *et al*. (2006). Identification and characterization of carbohydrate molecules in mammalian cells recognized by dengue virus type 2. *J Biochem.*, 139: 607–14.

Apte-Sengupta, S., Sirohi, D. and Kuhn, R.J. (2014). Coupling of replication and assembly in flaviviruses. *Curr. Opin. Virol*., 9: 134–142.

Artpradit, C., Robinson, L.N., Gavrilov, B.K. *et al*. (2013). Recognition of heparan sulfate by clinical strains of dengue virus serotype 1 using recombinant subviral particles. *Virus Res*., 176: 69–77.

Bäck, A.T. and Lundkvist, A. (2013). Dengue viruses - an overview. *Infect. Ecol. Epidemiol*., 3: 19839.

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L. *et al*. (2013). The global distribution and burden of dengue. *Nature*. 496(7446): 504–507.

Bryant, J.E., Calvert, A.E., Mesesan, K. *et al*. (2007). Glycosylation of the dengue 2 virus E protein at N67 is critical for virus growth in vitro but not for growth in intrathoracically inoculated *Aedes aegypti* mosquitoes. *Virology*, 366: 415–23.

Cao-Lormeau, V.M. (2009). Dengue viruses binding proteins from *Aedes aegypti* and Aedes polynesiensis salivary glands. *Virol J*., 6: 35.

Che, P., Tang, H. and Li, Q. (2013). The interaction between claudin-1 and dengue viral prM/M protein for its entry. *Virology*, 446: 303–13.

Chen, Y., Maguire, T., Hileman, R.E. *et al*. (1997). Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. *Nat Med*., 3: 866–71.

Chen, Y.C., Wang, S.Y. and King, C.C. (1999). Bacterial lipopolysaccharide inhibits dengue virus infection of primary human monocytes/macrophages by blockade of virus entry via a CD14-dependent mechanism. *J Virol*., 73: 2650–7.

Chin, J.F., Chu, J.J., Ng, M.L. (2007). The envelope glycoprotein domain III of dengue virus serotypes 1 and 2 inhibit virus entry. *Microbes Infect*., 9: 1–6.

Crill, W.D. and Roehrig, J.T. (2001). Monoclonal antibodies that bind to domain III of dengue virus E glycoprotein are the most efficient blockers of virus adsorption to Vero cells. *J Virol* 75: 7769–73.

Da Poian, A.T., Carneiro, F.A., Stauffer, F. (2005). Viral membrane fusion: is glycoprotein G of rhabdoviruses a representative of a new class of viral fusion proteins? *Braz J Med Biol Res.*, 38: 813–23.

Daecke, J., Fackler, O.T., Dittmar, M.T. *et al*. (2005). Involvement of clathrinmediated endocytosis in human immunodeficiency virus type 1 entry. *J Virol.*, 79: 1581–94.

Dejnirattisai, W., Webb, A.I., Chan, V. *et al*. (2011). Lectin switching during dengue virus infection. *J Infect Dis*., 203: 1775–83.

Duan, X., Lu, X., Li, J. *et al*. (2008). Novel binding between pre-membrane protein and vacuolar ATPase is required for efficient dengue virus secretion. *Biochem Bioph Res Co*., 373: 319–24.

Fibriansah, G., Ng, T.S., Kostyuchenko, V.A. *et al*. (2013). Structural changes in dengue virus when exposed to a temperature of 37 degrees C. *J Virol*., 87: 7585–92.

Fibriansah, G., Tan, J.L., Smith, S.A. *et al*. (2014). A potent anti-dengue human antibody preferentially recognizes the conformation of E protein monomers assembled on the virus surface. *EMBO Mol Med.*, 6: 358–71.

Freire, J.M., Veiga, A.S., Conceicao, T.M. *et al*. (2013b). Intracellular nucleic acid delivery by the supercharged dengue virus capsid protein. *PLoS One*, 8: e81450.

Freire, J.M., Veiga, A.S., de la Torre, B.G. *et al*. (2013a). Peptides as models for the structure and function of viral capsid proteins: Insights on dengue virus capsid. *Biopolymers*, 100:325–36.

Freire, J.M., Veiga, A.S., Rego de Figueiredo, I. *et al*. (2014). Nucleic acid delivery by cell penetrating peptides derived from dengue virus capsid protein: design and mechanism of action. *FEBS J.*, 281: 191–215.

Gao, F., Duan, X., Lu, X. *et al*. (2010). Novel binding between pre-membrane protein and claudin-1 is required for efficient dengue virus entry. *Biochem Bioph Res Co*., 391:952–7.

Germi, R., Crance, J.M., Garin, D. *et al*. (2002). Heparan sulfate-mediated binding of infectious dengue virus type 2 and yellow fever virus. *Virology*, 292: 162–8.

Grove, J., Marsh, M. (2011). The cell biology of receptor-mediated virus entry. *J Cell Biol.*, 195: 1071–82.

Gubler, D.J. (2006). Dengue/dengue haemorrhagic fever: history and current status. *Novartis Fdn Symp*, 277: 3–16.

Hacker, K., White, L., de Silva, A.M. (2009). N-linked glycans on dengue viruses grown in mammalian and insect cells. *J Gen Virol*., 90: 2097–106.

Harris, H.J., Davis, C., Mullins, J.G. *et al*. (2010). Claudin association with CD81 defines hepatitis C virus entry. *J Biol Chem.*, 285: 21092–102.

Harrison, S.C. (2008). Viral membrane fusion. *Nat Struct Mol Biol*., 15: 690–8.

Hawley, W.A., Reiter, P., Copeland, R.S., Pumpuni, C.B. and Craig, G.B. (1987). *Aedes albopictus* in North America: probable introduction in used tires from northern *Asia. Science*. 236(4805): 1114–1116

Hidari, K.I. and Suzuki, T. (2011). Dengue virus receptor. *Trop Med Health*., 39: 37–43.

Higa, L.M., Curi, B.M., Aguiar, R.S. *et al*. (2014). Modulation of alpha-enolase post-translational modifications by dengue virus: increased secretion of the basic isoforms in infected hepatic cells. *PLoS One*, 9:e88314.

Holmas, E.C. (1998). Molecular epidemiology and evolution of emerging infectious diseases. *Br Med Bull*., 54(3): 533–543

Hubner, W., McNerney, G.P., Chen, P. *et al*. (2009). Quantitative 3D video microscopy of HIV transfer across T cell virological synapses. *Science*, 323: 1743–7.

Hung, J.J., Hsieh, M.T., Young, M.J. *et al*. (2004). An external loop region of domain III of dengue virus type 2 envelope protein is involved in serotype-specific binding to mosquito but not mammalian cells. *J Virol*., 78: 378–88.

Hussain, T., Jamal, M., Rehman, T. and Andleeb, S. (2015). Dengue: pathogenesis, prevention and treatment – a mini review. *Adv Life Sci*., 2(3): 110–114.

Jemielity, S., Wang, J.J., Chan, Y.K. *et al*. (2013). TIM-family proteins promote infection of multiple enveloped viruses through virionassociated phosphatidylserine. *PLoS Pathog.*, 9: e1003232.

Jindadamrongwech, S., Smith, D.R. (2004). Virus Overlay Protein Binding Assay (VOPBA) reveals serotype specific heterogeneity of dengue virus binding proteins on HepG2 human liver cells. *Intervirology*, 47: 370–3.

Jindadamrongwech, S., Thepparit, C. and Smith, D.R. (2004). Identification of GRP 78 (BiP) as a liver cell expressed receptor element for dengue virus serotype 2. *Arch Virol*, 149: 915–27.

Johnson, A.J., Guirakhoo, F. and Roehrig, J.T. (1994). The envelope glycoproteins of dengue 1 and dengue 2 viruses grown in mosquito cells differ in their utilization of potential glycosylation sites. *Virology*, 203: 241–9.

Junjhon, J., Edwards, T.J., Utaipat, U. *et al*. (2010). Influence of pr-M cleavage on the heterogeneity of extracellular dengue virus particles. *J Virol*, 84: 8353–8.

Klein, D.E., Choi, J.L. and Harrison, S.C. (2013). Structure of a dengue virus envelope protein late-stage fusion intermediate. *J Virol*, 87: 2287–93.

Kobayashi, T., Beuchat, M.H. and Chevallier, J. *et al*. (2002). Separation and characterization of late endosomal membrane domains. *J Biol Chem*, 277: 32157–64.

Krishnan, M.N., Sukumaran, B., Pal, U. *et al*. (2007). Rab 5 is required for the cellular entry of dengue and West Nile viruses. *J Virol*, 81: 4881–5.

Kuadkitkan, A., Wikan, N. and Fongsaran, C. *et al*. (2010). Identification and characterization of prohibitin as a receptor protein mediating DENV-2 entry into insect cells. *Virology*, 406: 149–61.

Kumar, A., Arya, H., Singh, A. P., Singh, S., Sharma, S., & Singh, K. (2023). Surveillance of Aedes Diversity, Seasonal Prevalence and Habitat Characterization in Bulandshahr, Utter Pradesh, India. *Journal of Science Innovations and Nature of Earth*, 3(1): 04-10.

Lee, E., Wright, P.J., Davidson, A. *et al*. (2008). Virulence attenuation of Dengue virus due to augmented glycosaminoglycan-binding affinity and restriction in extraneural dissemination. *J Gen Virol*., 87: 2791–801.

Li, L., Lok, S.M., Yu, I.M. *et al*. (2008). The flavivirus precursor membraneenvelope protein complex: structure and maturation. Science, 319: 1830–4.

Liu, S., Yang, W., Shen, L. *et al*. (2009). Tight junction proteins claudin-1 and occludin control hepatitis C virus entry and are downregulated during infection to prevent superinfection. *J Virol.*, 83: 2011–4.

Luo, D., Vasudevan, S.G. and Lescar, J. (2015). The flavivirus NS2B-NS3 protease-helicase as a target for antiviral drug development. *Antiviral Res*. 118: 148–158.

Marovich, M., Grouard-Vogel, G., Louder, M. *et al*. (2001). Human dendritic cells as targets of dengue virus infection. *J Invest Derm Symp*, 6: 219–24.

Martinez-Barragan, J.J., del Angel, R.M. (2001). Identification of a putative coreceptor on Vero cells that

participates in dengue 4 virus infection. *J Virol.*, 75: 7818–27.

McNaughton, B.R., Cronican, J.J., Thompson, D.B. *et al*. (2009). Mammalian cell penetration, siRNA transfection, and DNA transfection by supercharged proteins. P Natl Acad Sci USA, 106: 6111–6.

Meertens, L., Bertaux, C., Cukierman, L. *et al*. (2008). The tight junction proteins claudin-1, -6, and -9 are entry cofactors for hepatitis C virus. *J Virol*, 82: 3555–60.

Meertens, L., Carnec, X., Lecoin, M.P. *et al*. (2012). The TIM and TAM families of phosphatidylserine receptors mediate dengue virus entry. *Cell Host Microbe*, 12: 544–57.

Melikyan, G.B. (2014). HIV entry: a game of hide-and-fuse? *Curr Opin Virol*, 4: 1–7.

Mercado-Curiel, R.F., Black, W.Ct., Munoz, Mde L. (2008). A dengue receptor as possible genetic marker of vector competence in *Aedes aegypti*. BMC Microbiol, 8: 118.

Mercado-Curiel, R.F., Esquinca-Aviles, H.A., Tovar, R. *et al*. (2006). The four serotypes of dengue recognize the same putative receptors in *Aedes aegypti* midgut and Ae. albopictus cells. *BMC Microbiol*, 6: 85.

Mercer, J., Helenius, A. (2008). Vaccinia virus uses macropinocytosis and apoptotic mimicry to enter host cells. *Science*, 320: 531–5.

Miller, J.L., de Wet, B.J., Martinez-Pomares, L. *et al*. (2008). The mannose receptor mediates dengue virus infection of macrophages. *PLoS Pathog*, 4: e17.

Miyauchi, K., Kim, Y., Latinovic, O. *et al*. (2009). HIV enters cells via endocytosis and dynamin-dependent fusion with endosomes. *Cell*, 137: 433–44.

Modis, Y., Ogata, S., Clements, D. *et al*. (2003). A ligand-binding pocket in the dengue virus envelope glycoprotein. *P Natl Acad Sci USA*,100: 6986–91.

Modis, Y., Ogata, S., Clements, D. *et al*. (2004). Structure of the dengue virus envelope protein after membrane fusion. *Nature*, 427: 313–9.

Modis, Y., Ogata, S., Clements, D. *et al*. (2005). Variable surface epitopes in the crystal structure of dengue virus type 3 envelope glycoprotein. *J Virol.*, 79: 1223–31.

Modis, Y. (2014). Relating structure to evolution in class II viral membrane fusion proteins. *Curr Opin Virol.*, 5: 34–41.

Mondotte, J.A., Lozach, P.Y., Amara, A. *et al*. (2007). Essential role of dengue virus envelope protein N glycosylation at asparagine-67 during viral propagation. *J Virol*, 81: 7136–48.

Mosso, C., Galvan-Mendoza, I.J., Ludert, J.E. *et al*. (2008). Endocytic pathway followed by dengue virus to infect the mosquito cell line C6/36 HT. Virology, 378: 193–9.

Mukhopadhyay, S., Kuhn, R.J., Rossmann, M.G. (2005). A structural perspective of the flavivirus life cycle. *Nat Rev Microbiol*., 3: 13–22.

Munoz Mde, L., Limon-Camacho, G., Tovar, R. *et al*. (2013). Proteomic identification of dengue virus binding proteins in *Aedes aegypti* mosquitoes and *Aedes albopictus* cells. *Biomed Res Int*., 2013: 875958.

Munoz, M.L., Cisneros, A., Cruz, J. *et al*. (1998). Putative dengue virus receptors from mosquito cells. *FEMS Microbiol Lett.*, 168: 251–8.

Mustafa, M.S., Rasotgi, V., Jain, S. and Gupta, V. (2015). Discovery of fifth serotype of dengue virus (DENV-5): a new public health dilemma in dengue control. *Med J Armed Forces India*, 71(1): 67–70.

Mutheneni, S.R., Morse, A.P., Caminade, C. and Upadhyayula, S.M. (2017). Dengue burden in India: recent trends and importance of climatic parameters. *Emerg Microbes Infect*, 6(8): e70

Navarro-Sanchez, E., Altmeyer, R., Amara, A. *et al*. (2003). Dendritic-cellspecific ICAM3-grabbing non-integrin is essential for the productive infection of human dendritic cells by mosquito-cellderived dengue viruses. *EMBO Rep.*, 4: 723–8.

Ng, W.C., Soto-Acosta, R., Bradrick, S.S., Garcia-Blanco, M.A. and Ooi, E.E. (2017). The 5' and 3' untranslated regions of the flaviviral genome. *Viruses* 9(6): 137.

Nour, A.M., Li, Y., Wolenski, J. *et al*. (2013). Viral membrane fusion and nucleocapsid delivery into the cytoplasm are distinct events in some flaviviruses. *PLoS Pathog*,, 9: e1003585.

Obi, J.O., Gutié rrez-Barbosa, H., Chua, J.V. and Deredge, D.J. (2021). Current trends and limitations in dengue antiviral research. *Trop. Med. Infect. Dis.*, 6(4): 180.

Padilla-Parra, S., Marin, M., Gahlaut, N. *et al*. (2013). Fusion of mature HIV-1 particles leads to complete release of a gag-GFPbased content marker and raises the intraviral pH. *PLoS One*, 8: e71002.

Perera, R., Kuhn, R.J. (2014). Structural proteomics of dengue virus. *Curr Opin Microbiol.*, 11: 369–77.

Perera-Lecoin, M., Meertens, L., Carnec, X. *et al*. (2014). Flavivirus entry receptors: an update. *Viruses*, 6: 69–88.

Pierson, T.C., Kielian, M. (2013). Flaviviruses: braking the entering. *Curr Opin Virol*, 3:3–12.

Pokidysheva, E., Zhang, Y., Battisti, A.J. *et al*. (2006). Cryo-EM reconstruction of dengue virus in complex with the carbohydrate recognition domain of DC-SIGN. *Cell*, 2006; 124: 485–93.

Prestwood, T.R., Prigozhin, D.M., Sharar, K.L. *et al*. (2008). A mouse-passaged dengue virus strain with reduced affinity for heparan sulfate causes severe disease in mice by establishing increased systemic viral loads. *J Virol* 82: 8411–21.

Ramos-Castaneda, J., Imbert, J.L., Barron, B.L. *et al*. (1997). A 65-kDa trypsinsensible membrane cell protein as a possible receptor for dengue virus in cultured neuroblastoma cells. *J Neurovirol.*, 3: 435–40.

Reyes-del, Valle J., Chavez-Salinas, S., Medina, F. *et al*. (2005). Heat shock protein 90 and heat shock protein 70 are components of dengue virus receptor complex in human cells. *J Virol*, 79: 4557–67.

Reyes-del Valle, J., del Angel, R.M. (2004). Isolation of putative dengue virus receptor molecules by affinity chromatography using a recombinant E protein ligand. *J Virol Methods.*, 116: 95–102.

Rodenhuis-Zybert, I.A., van der Schaar, H.M., da Silva Voorham, J.M. *et al*. (2010). Immature dengue virus: a veiled pathogen? PLoS Pathog, 6: e1000718.

Rodenhuis-Zybert, I.A., Wilschut, J., Smit, J.M. (2010). Dengue virus life cycle: viral and host factors modulating infectivity. *Cell Mol Life Sci.*, 67: 2773–86.

Rodenhuis-Zybert, I.A., Wilschut, J., Smit, J.M. (2011). Partial maturation: an immune-evasion strategy of dengue virus? *Trends Microbiol*, 19: 248–54.

Rodenhuis-Zybert, I.A., van der Schaar, H.M., da Silva Voorham, J.M., van der Ende-Metselaar, H., Lei, H.Y., Wilschut, J. *et al*. (2010). Immature dengue virus: a veiled pathogen? *PloS Pathog*. 6(1): e1000718.

Sakoonwatanyoo, P., Boonsanay, V., Smith, D.R. (2006). Growth and production of the dengue virus in C6/36 cells and identification of a laminin-binding protein as a candidate serotype 3 and 4 receptor protein. Intervirology, 49: 161–72.

Sakuntabhai, A., Turbpaiboon, C., Casademont, I. *et al*. (2005). A variant in the CD209 promoter is associated with severity of dengue disease. *Nat Genet.*, 37: 507–13.

Salas-Benito, J.S., delAngel, R.M. (1997). Identification of two surface proteins from C6/36 cells that bind dengue type 4 virus. J Virol 1997;71:7246–52.

Shapiro J, Sciaky N, Lee J, *et al*. Localization of endogenous furin in cultured cell lines. J Histochem Cytochem 1997;45:3–12.

Smit JM, Moesker B, Rodenhuis-Zybert I, *et al*. Flavivirus cell entry and membrane fusion. Viruses 2011;3:160–71.

Suksanpaisan L, Susantad T, Smith DR. Characterization of dengue virus entry into HepG2 cells. J Biomed Sci 2009; 16:17.

Suomalainen M, Greber UF. Uncoating of non-enveloped viruses. Curr Opin Virol 2013;3:27–33.

Tassaneetrithep B, Burgess TH, Granelli-Piperno A, *et al*. DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. J Exp Med 2003;197:823–9.

Thepparit C, Phoolcharoen W, Suksanpaisan L, *et al*. Internalization and propagation of the dengue virus in human hepatoma (HepG2) cells. Intervirology 2004;47:78–86.

Thepparit C, Smith DR. Serotype-specific entry of dengue virus into liver cells: identification of the 37-kilodalton/67- kilodalton high-affinity laminin receptor as a dengue virus serotype 1 receptor. J Virol 2004;78:12647–56.

Thompson DB, Cronican JJ, Liu DR. Engineering and identifying supercharged proteins for macromolecule delivery into mammalian cells. Methods Enzymol 2012;503: 293–319.

Upanan S, Kuadkitkan A, Smith DR. Identification of dengue virus binding proteins using affinity chromatography. J Virol Methods 2008;151:325–8.

Usme-Ciro JA, Campillo-Pedroza N, Almazan F, *et al*. Cytoplasmic RNA viruses as potential vehicles for the delivery of therapeutic small RNAs. Virol J 2013;10:185.

van der Schaar HM, Rust MJ, Chen C, *et al*. Dissecting the cell entry pathway of dengue virus by single-particle tracking in living cells. *PLoS Pathog*, 4: e1000244.

van der Schaar, H.M., Rust, M.J., Waarts, B.L. *et al*. (2007). Characterization of the early events in dengue virus cell entry by biochemical assays and single-virus tracking. *J Virol*., 81: 12019–28.

van Meer, G., Voelker, D.R., Feigenson, G.W. (2008). Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol.*, 9: 112–24.

Vancini, R., Kramer, L.D., Ribeiro, M. *et al*. (2013). Flavivirus infection from mosquitoes in vitro reveals cell entry at the plasma membrane. *Virology*, 435: 406–14.

Watterson, D., Kobe, B., Young, P.R. (2012). Residues in domain III of the dengue virus envelope glycoprotein involved in cell-surface glycosaminoglycan binding. *J Gen Virol.*, 93: 72–82.

Wei, H.Y., Jiang, L.F., Fang, D.Y. *et al*. (2003). Dengue virus type 2 infects human endothelial cells through binding of the viral envelope glycoprotein to cell surface polypeptides. *J Gen Virol.*, 84: 3095–8.

Whitehead, S.S., Blaney, J.E., Durbin, A.P. and Murphy, B.R. (2007). Prospects for a dengue virus vaccine. *Nat. Rev. Microbiol*. 5(7): 518–528.

Wichit, S., Jittmittraphap, A., Hidari, K.I. *et al*. (2011). Dengue virus type 2 recognizes the carbohydrate moiety of neutral glycosphingolipids in mammalian and mosquito cells. *Microbiol Immunol*, 55: 135–40.

Wu, S.J., Grouard-Vogel, G., Sun, W. *et al*. (2000). Human skin Langerhans cells are targets of dengue virus infection. *Nat Med.*, 6: 816–20.

Yazi Mendoza, M., Salas-Benito, J.S., Lanz-Mendoza, H. *et al*. (2002). A putative receptor for dengue virus in mosquito tissues: localization of a 45-kDa glycoprotein. *Am J Trop Med Hyg*., 67: 76–84.

Zaitseva, E., Yang, S.T., Melikov, K. *et al*. (2010). Dengue virus ensures its fusion in late endosomes using compartment-specific lipids. *PLoS Pathog*, 6:e1001131.

Zhang, X., Ge, P., Yu, X. *et al*. (2013a). Cryo-EM structure of the mature dengue virus at 3.5-A resolution. *Nat Struct Mol Biol.*, 20: 105–10.

Zhang, Y., Zhang, W., Ogata, S. *et al*. (2004). Conformational changes of the flavivirus E glycoprotein. *Structure*, 12: 1607–18.

Zheng, A., Umashankar, M., Kielian, M. (2010). *In vitro* and *in vivo* studies identify important features of dengue virus pr-E protein interactions. *PLoS Pathog*, 6: e1001157.

Zhong, P., Agosto, L.M., Munro, J.B. *et al*. (2013). Cell-to-cell transmission of viruses. *Curr Opin Virol*, 3: 44–50.

Zou, G., Chen, Y.L., Dong, H., Lim, C.C., Yap, L.J., Yau, Y.H. *et al*. (2011). Functional analysis of two cavities in flavivirus NS5 polymerase. *J. Biol. Chem*. 286(16): 14362–14372.

Zybert, I.A., van der Ende-Metselaar, H., Wilschut, J. *et al*. (2008). Functional importance of dengue virus maturation: infectious properties of immature virions. *J Gen Virol.*, 89: 3047–51.

Cite this article:
Anil Kumar, Hridayesh Arya, KalpanaSoni, Prveen, Vishan Kumar, Ajay Pratap Singh, Surbhi Mittal, Manish Maheshwari, Prem Sagar, Anand Pratap Singh, Sonal Singh and Keshav Singh, 2023, "A Review on the receptors and Pathways used by the Dengue Virus to Infect Host Cells" ***Journal of Science Innovations and Nature of Earth***, Vol. 3(3), page-20-32
www.doi.org/10.59436/jsiane.com/archives3/3/96