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# Coupling of replication and assembly in flaviviruses

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Flaviviruses affect hundreds of millions of people each year causing tremendous morbidity and mortality worldwide. This genus includes significant human pathogens such as dengue, West Nile, yellow fever, tick-borne encephalitis and Japanese encephalitis virus among many others. The disease caused by these viruses can range from febrile illness to hemorrhagic fever and encephalitis. A deeper understanding of the virus life cycle is required to foster development of antivirals and vaccines, which are an urgent need for many flaviviruses, especially dengue. The focus of this review is to summarize our current knowledge of flaviviral replication and assembly, the proteins and lipids involved therein, and how these processes are coordinated for efficient virus production.

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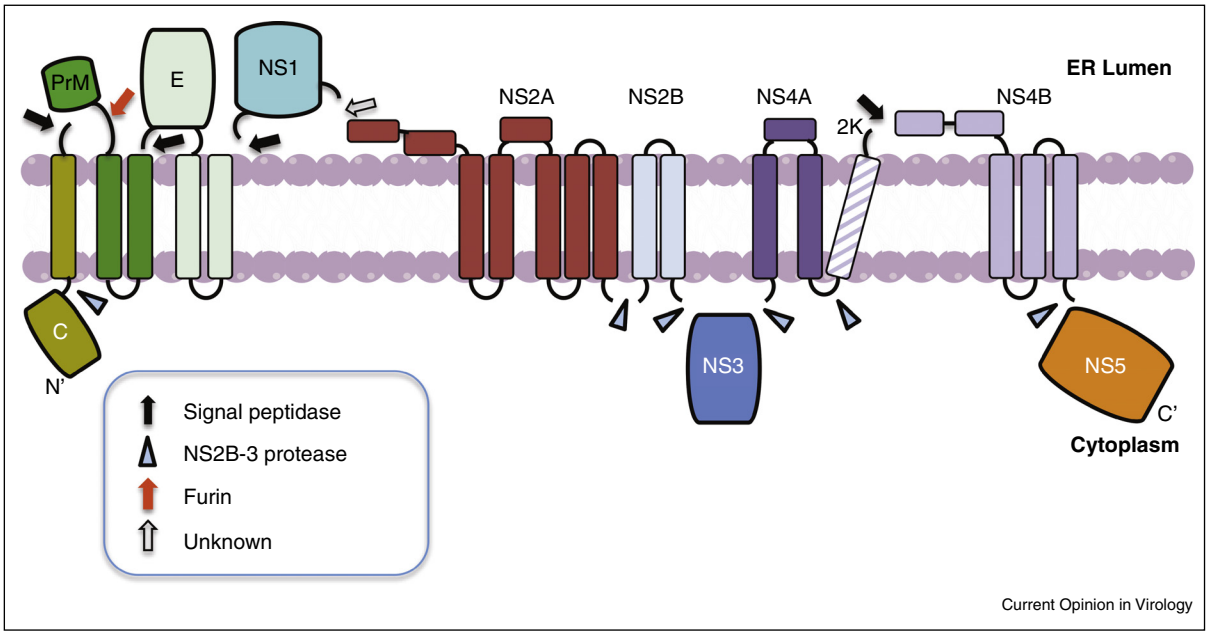
## Introduction

Flaviviruses (Family — *Flaviviridae*) are one of the major causes of arthropod-borne illness in humans. Flavivirus pathogenesis ranges from mild illness such as fever, rash and joint pain, to more severe symptoms such as hemorrhagic fever and fatal encephalitis. The flavivirus genus consists of more than 70 enveloped, positive-strand RNA viruses including yellow fever virus (YFV), dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV) and tick-borne encephalitis virus (TBEV). Altogether flaviviruses affect hundreds of millions of individuals every year. Despite the availability of vaccines against YFV, JEV and TBEV, diseases resulting from these viruses are still prevalent worldwide. Protective vaccines or therapies are not yet available against the more pathogenic flaviviruses and prevention from insect bites remains the major defense against some of these viruses. Therefore, a better understanding of the flavivirus life cycle is essential in order to develop effective strategies for antiviral intervention and for development of novel vaccines.

The virus enters host cells by receptor-mediated endocytosis and the ~11 kb positive-sense RNA genome gains entry into the cytoplasm by viral glycoprotein-mediated membrane fusion. Flavivirus replication begins when the genome is recognized as messenger RNA and translated by host cell machinery to yield a single polyprotein. The polyprotein is co-translationally and post-translationally cleaved by viral and cellular proteases into 10 gene products (Figure 1). The structural proteins capsid (C), precursor membrane (prM/M) and envelope (E) are incorporated into the virion, whereas the non-structural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 serve to coordinate the intracellular aspects of virus replication, assembly and modulation of host defense mechanisms. NS1 is essential for virus replication and inhibition of complement-mediated immune response [1]. NS3 contains serine protease, Nucleoside 5′ triphosphatase (NTPase), RNA helicase, and 5′ RNA triphosphatase (RTPase) activities, while NS2B serves as a cofactor for the protease activity of NS3. NS5 contains methyltransferase and RNA-dependent RNA polymerase (RdRp) domains required for genome replication and capping of nascent RNA [2]. Three non-enzymatic, integral membrane proteins NS2A, NS4A and NS4B are poorly understood. NS2A is required for virus replication and assembly [3–5]. NS4A induces membrane rearrangement [6,7] and autophagy to enhance viral replication [8], whereas NS4B modulates host immune response by suppressing the α/β interferon signaling and the helicase activity of NS3 [9,10].

Polyprotein processing into component proteins takes place on ER membranes (Figure 1). The replication complex (RC) is formed on modified ER membranes where negative-sense RNA is copied from the genomic RNA template. The process of RNA synthesis is asymmetric favoring positive-sense RNA [2]. The RNA genome then interacts with C protein, which buds through the glycoprotein-containing ER membranes as immature virus particles into the lumen. The immature virus particles then go through maturation steps in the ER and Golgi complex and are released as mature particles from the cell. The biogenesis of the RC and its assembly involve modifications of cellular metabolic and structural components. This review presents an overview of the current knowledge of interactions between the viral and cellular components to promote cellular changes required for replication and assembly and how these two processes are coupled. Replication and assembly are important

**Figure 1**




Flavivirus polyprotein processing. Translation of the viral genome results in a polyprotein, which is cleaved by cellular and viral proteases. The proposed topologies of the viral proteins with respect to the ER lumen and cytoplasm and the proteases responsible for their cleavages are indicated. The membrane bilayer is shown in pink, transmembrane domains for individual proteins are shown as cylinders spanning the membrane and connecting loops are shown as lines. Black, blue and red arrow/arrowheads represent signal peptidase, NS2B-3 protease and furin protease cleavage sites respectively. Open arrow represents a protease cleavage site that required additional characterization.

aspects of the virus life cycle that are targeted for future antiviral development.

## Flavivirus translation and replication

### Virus-induced membrane rearrangements

It is now established that flavivirus replication and viral RNA synthesis occurs on an extended network of modified ER membranes. At least three distinct membranous structures are found in flavivirus-infected cells: membranous sacs or vesicle packets (Vp), membrane vesicles (Ve) and convoluted membranes (CM) (Figure 2) [11–13,14••,15•,16•]. Vps are small clusters of Ve formed by modification of ER membranes and used as sites of replication by the virus [13,14••]. Ve are open to the cytoplasm via a small neck. The CMs are suggested to form the sites of translation and polyprotein processing and/or storage sites for viral proteins [14••,15•]. These structures are formed by membrane remodeling that can be induced by changes in lipid composition, influence of integral membrane proteins, activities of cytoskeletal protein and microtubule motors, scaffolding by peripheral and integral membrane proteins [17]. Certain lipids such as lysophosphatidic acid and phosphatidic acid favor negative membrane curvature [18]. Similarly, enzymes such as flippase can also introduce membrane asymmetry [19].

It is still unclear what triggers the membrane modifications in flavivirus infection, viral proteins along with modulation of host lipid metabolism and cytoskeletal components are involved. Expression of KUNV and DENV NS4A containing the uncleaved carboxyl-terminal transmembrane domain, 2K, induced ER membrane rearrangements, while a role of NS4B in membrane rearrangement has also been suggested [6,7,20]. The amino terminal amphipathic helix of NS4A induces homo-oligomerization and is also predicted to form a scaffold in the membrane to induce membrane bending [21]. Additionally, NS4A of DENV was shown to rearrange vimentin intermediate filaments at perinuclear sites. This rearrangement of vimentin along with vimentin phosphorylation was implicated in enrichment of RCs [22]. KUNV NS2A has been implicated in membrane rearrangements, as a mutation at residue 59 (I59A) blocked membrane rearrangements and virus assembly [5]. Recently, NS1 was also shown to have membrane remodeling ability [23]. When purified NS1 was mixed with liposomes rich in cholesterol and phosphatidyl serine, they were fragmented into small nanoparticles suggesting that NS1 possesses an ability to remodel lipid membranes [23].

Comparable membranous structures were reported in both insect and mammalian cells infected with flaviviruses. However, CMs were not observed in DENV-infected insect cells [15•]. A higher number of closed ended tubular structures were seen in the TBEV-infected