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Review

Role of Endoplasmic Reticulum-Associated Proteins in Flavivirus Replication and Assembly Complexes

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Abstract: Flavivirus replication in host cells requires the formation of replication and assembly complexes on the cytoplasmic side of the endoplasmic reticulum (ER) membrane. These complexes consist of an ER membrane, viral proteins, and host proteins. Genome-wide investigations have identified a number of ER multiprotein complexes as vital factors for flavivirus replication. The detailed mechanisms of the role of ER complexes in flavivirus replication are still largely elusive. This review highlights the fact that the ER multiprotein complexes are crucial for the formation of flavivirus replication and assembly complexes, and the ER complexes could be considered as a target for developing successful broad-spectrum anti-flavivirus drugs.

Keywords: flavivirus; virus replication complex; endoplasmic reticulum; host factors

1. Introduction

Members of flavivirus genus are the most important arthropod-borne viruses causing disease in humans. This genus includes pathogens of public health importance including the West Nile virus (WNV), Japanese encephalitis virus (JEV), dengue virus (DENV), yellow fever virus (YFV), tick-borne encephalitis virus (TBEV), and Zika virus (ZIKV) [1,2]. Flaviviruses continue to spread and cause human disease in new areas of the world [3]. No effective therapies exist for treating individuals with flavivirus infections. The lack of specific therapeutics for flavivirus infections imparts a pressing need to identify the viral and host factors in flavivirus replication and disease outcome.

Flaviviruses infect the host cells by binding with virus receptors on the cell membrane [4]. The direct interaction of the virus with the specific receptor induces clathrin-mediated endocytosis, a major endocytic process by which the cells uptake nutrients from the surrounding environment [5–7]. The acidic environment in the cellular endosomes facilitates the envelope disassembly and release viral genome, a capped, positive-sense, single-stranded, 11 kb RNA to the cytosol. Once the viral RNA binds to the ribosomes by 5′-cap structure, the translation process produces a viral polyprotein anchored to the ER membrane [4]. The viral polyprotein undergoes multi-sites cleavage by viral and cellular proteases into three structural proteins (Capsid [C], pre-membrane [prM], and Envelope [E]) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [8]. Viral structural proteins construct new virions by C protein-viral RNA binding, covered with prM and E proteins [9]. Viral non-structural proteins are responsible for viral replication, attenuation of host immune response, manipulating cell structures and functions, and other yet to be known interactions with host proteins [10,11].

Viral proteins alter the endoplasmic reticulum (ER) membrane to generate new structures called vesicle packets (VPs), containing viral replication and assembly complexes [12]. The mechanisms underlying virus replication and assembly on the ER membrane remain to be fully understood. This review highlights the role of ER proteins in the formation of flavivirus replication and assembly complexes. To date, no antiviral drugs are available to combat infections caused by flaviviruses. Thus,

targeting the host factors that have important roles in regulating the formation and stabilization of flavivirus replication and assembly complexes represents a new therapeutic approach for developing anti-flavivirus drugs.

2. Flavivirus Replication and Assembly Complexes

Flaviviruses replicate in host cells on the cytoplasmic side of the ER membrane. The ER membrane undergoes an extensive re-arrangement after releasing viral proteins, creating different structures. Flavivirus proteins induce a remodeling of the ER membrane into three distinct structures: vesicle packets (VPs), convoluted membranes (CM), and membrane vesicles (Ve) [13–16]. The small viral non-structural proteins serve as the scaffold for the membrane-associated replication complex [17]. NS1, NS2A, NS2B, NS4A, and NS4B are characterized as viral proteins that have the ability to remodel the ER membrane [18–20]. For example, ZIKV NS2A has a single segment that traverses the ER membrane and six segments that peripherally associate with the ER membrane, which are essential for viral RNA synthesis and virion assembly [21]. The formations of NS4A and NS4B homo-oligomers and hetero-oligomers and NS1 homo-dimers are necessary to remodel the ER membrane [22]. However, it is unclear how the viral proteins are repositioned in the ER-lumen or translocated to the cytoplasm during the rearrangement of the ER membrane.

Flavivirus replication depends on the enzymatic activities of NS3 and NS5 proteins that form a replication complex. NS3 has a protease, helicase, adenosine triphosphatase (ATPase), and RNA 5′ triphosphatase (RTPase) activities [23–26] and NS5 has methyltransferase and RNA-dependent RNA polymerase activities [27–29]. NS3 helicase and ATPase activities are required for unwinding double-stranded RNA utilizing the chemical energy derived from ATP hydrolysis [30]. Flavivirus capsid protein associates with the ER membrane and distributes on the surface of the lipids droplets (LDs) in the cytoplasm [31]. ER membrane-associated capsid protein distributes close to the exit of RNA replication sites (the vesicle packets) [13,16,32]. Viral NS3 plays an important role in the timing of RNA synthesis (helicase activity) and capsid protein maturation (cleave PreM-C junction as protease). The timing of capsid protein maturation and localization close to RNA synthesis are important for the capsid protein function as a RNA chaperone during virus assembly [33–37] (Figure 1).

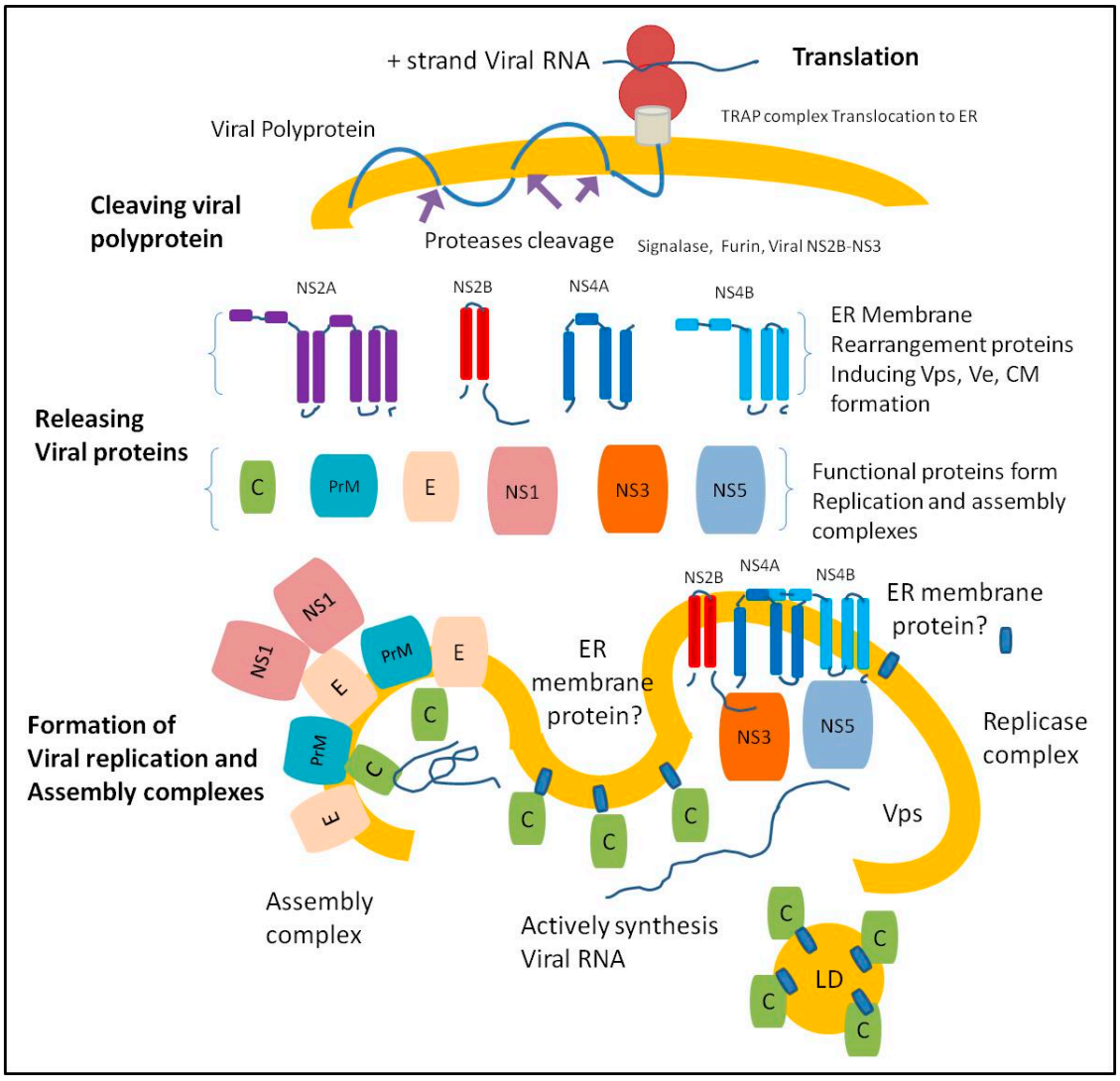

Figure 1. The endoplasmic reticulum (ER) membrane undergoes extensive re-arrangement after releasing viral proteins creating distinct structures. Once the ER-associated ribosome translates viral RNA, the ER complex called mammalian translocon-associated protein (TRAP) translocates the newly synthesized viral polyprotein to the ER lumen and the translocated polyprotein integrates into the ER membrane [38,39]. The cleaving of the viral polyprotein occurs when the host cell proteases (furin and signalase) gain access to the cleaving sites of the viral polyprotein, releasing viral protease units (NS2B and NS3). The central 40 amino-acid region of the NS2B co-factor is crucial to the NS3 protease function. The NS2B-NS3 serine protease cleaves viral polyprotein at various sites to release the structural and non-structural proteins [40–47].

3. ER proteins Required for Flavivirus Replication and Assembly

CRISPR and Genomic RNA interference screens indicated that flaviviruses require overlapping as well as specific host factors to promote viral infection. The Genome-wide loss of function studies identified various ER proteins crucial for virus replication. These proteins are involved in the regulation of the stress response, protein modification and degradation, RNA translation, signal transduction, and apoptosis [48–53].

1. DNAJ homolog subfamily C member 14 (DNAJC14)

DNAJC14 is the heat shock protein40 (Hsp40) chaperone (also named DRIP78, Jiv, and LIP6) that modulates the dopamine D1 receptor transport from the ER to the plasma membrane [54].

DNAJC14 contains a conserved 70-amino-acid J domain that interacts with Hsp70 family members to stimulate ATP hydrolysis during chaperone activity [55]. Interestingly, the interaction between DNAJC14 and flavivirus non-structural proteins altered the properties of the ER membrane and resulted in the formation of the protein scaffold that maintains the viral replication complex [56]. Thus, DNAJC14 is a vital ER-associated chaperone required for the integration of the flavivirus replication complex to a specific ER membrane location [56,57]. Furthermore, DNAJC14 plays a central role in ER stress-associated unconventional protein secretion [58] that are induced during virus infection. It is also shown to co-localize with dsRNA within the YFV replication complex. It has been reported that endogenous levels of DNAJC14 are vital for YFV replication [57]. A similar role of DNAJC14 has recently been observed in the RNA replication of the bovine viral diarrhea virus (BVDV) [59]. Therefore, DNAJC14 is a key host cell factor for flavivirus replication.

2. Hrd1 complex

The endoplasmic reticulum-associated degradation (ERAD) pathway includes misfolded protein recognition, translocation, ubiquitylation, and cytoplasmic proteasomal degradation. When the nascent polypeptide is synthesized by the ribosomes, it will integrate into the ER membrane and dislocate to the ER lumen [60]. ER chaperones process protein folding and assemble the subunits in functional and secreted multi-domain proteins. Viral proteins like NS3 and NS5 are multi-domain and multi-functional enzymes that may require refolding processing by cellular chaperones. Thus, the accumulation of viral proteins in the ER lumen induces the unfolded protein response (UPR) to ER stress and up-regulate cellular chaperones expression to expedite viral protein refolding [61,62]. The misfolded proteins and orphan subunits are subjected to the Hrd1 complex for translocation, ubiquitylation, and proteasomal degradation [63,64]. The Hrd1 complex consists of ER luminal lectins, chaperones, ER membrane proteins, and cytoplasmic proteins [65].

Hrd1 protein has emerged as a critical host factor required for flaviviruses replication [48–51,66,67]. The inhibition of protein translocation from ER to the cytosol or inhibiting the ER chaperone grp94 by small molecule compounds led to a significant decrease in DENV and ZIKV replication [68,69]. Blocking proteasomal degradation by the proteasome inhibitors Bortezomib significantly decreased DENV and ZIKV replication in vitro and attenuated the infection in vivo [70,71]. Genomic and proteomic screening methods have demonstrated that DENV and WNV replication requires Hrd1, Derlin2, and Ube2j1 proteins of the Hrd1 complex [48]. Proteins in the Hrd1 complex have been reported to interact with several viral proteins. For example, Derlin2 interacts with NS5 of DENV and ZIKV and with NS4B of ZIKV [51,67] (Table 1). Thus, targeting Hrd1 complex could be a new avenue in developing novel anti- flavivirus drugs [68,69].

Table 1. Hrd1 complex proteins required for flaviviruses replication.

Flavivirus	Hrd1 Subunits Required for Virus Replication	References
Dengue virus	SEL1L, AUP1, DERL2, UBE2J1, EMC2	[51,67]
West Nile virus	Hrd1, DERL2, SEL1L, UBE2J1, UBE2G2,	[48,49]
Zika virus	DERL2, AUP1	[51]
Japanese encephalitis virus	GRP78 (Bip)	[72]

3. Oligosaccharyltransferase (OST) complex

The ER-associated oligosaccharyltransferase (OST) complex catalyzes the N-linked glycosylation of newly synthesized proteins. The two OST protein isoforms, which are multiprotein complexes, are composed of a catalytic subunit, STT3A or STT3B, and accessory subunits [73]. The OST complex is associated with flavivirus replication through the interaction with viral non-structural proteins. The deletion of OST subunits resulted in a >99% reduction of flavivirus infections in cell culture [50].

The catalytic function of the OST complex is not required for DENV replication, suggesting that the complex may have a structural role in the formation of the replication complex [47]. DENV RNA replication is independent on the presence of both OST isoforms, while ZIKV, YFV, and WNV replication exclusively depend on the STT3A OST complex, demonstrating differences in the requirement for OST complex variants among flaviviruses [74]. The OST complex activity is inhibited by a small molecule compound named NGI-1 [75]. This OST inhibitor exhibited anti-viral activity against flaviviruses indicating that the OST cellular pathway could be exploited for anti-viral drug discovery [74].

4. Reticulon 3 (RTN 3.1A)

In mammals, the Reticulon protein family comprises of RTN1, RTN2, RTN3, and RTN4, which are widely expressed in most tissues, especially in human and mouse brains [76–78]. The RTN 3.1A, ER-membrane proteins reside primarily within the ER and Golgi apparatus [76]. It is known that RTN3.1A facilitates WNV, DENV, and ZIKV replication via direct or indirect interaction with viral NS4A to facilitate replication complex formation [79]. The absence of RTN3.1A promotes the degradation of the viral NS4A protein, eventually disturbing the formation of the replication complex and the production of viral particles [79].

5. ER membrane complex (EMC)

Several studies have reported the critical role of the ER membrane complex (EMC) in flavivirus replication [50,52,53]. It has been proposed that EMC serves as an ER chaperone for processing the multi-pass transmembrane proteins [80–82] such as flavivirus membrane proteins, NS2A, NS2B, NS4A, and NS4B, which are necessary for virus replication. It has been reported that EMC promotes the biogenesis of DENV and ZIKV non-structural multi-pass transmembrane proteins NS4A and NS4B [83]. Furthermore, EMC serves as an insertase for selective tail-anchored membrane proteins [84]. The EMC has been found to bind to NS4B and colocalized with the DENV replication organelle [83].

4. ER-Associated Proteins Critical for Virus Assembly and Egress

The role of ER protein complexes in flavivirus assembly and egress has not been extensively investigated. Nevertheless, some studies showed that individual ER proteins have a significant role in flavivirus assembly and egress. It has been reported that DDX56, a host helicase facilitates WNV assembly by transferring the newly synthesized viral RNA to the assembly site through direct binding to the capsid [85]. Other host proteins such as Src Kinases also represent crucial factors for DENV and WNV assembly and egress by facilitating virus passing from ER to the Golgi [86]. KDEL receptors (KDELR), which cycle between the ER and Golgi apparatus is vital for DENV trafficking from ER to Golgi by interacting with viral prM [87]. Other factors that are essential for DENV, WNV, and JEV assembly and egress includes Ras-related in brain protein (Rab8b), endosomal sorting complex that is required for transport (ESCRT), and the ADP-ribosylation proteins (Arf4/5). [88–91].

5. Conclusions

Flaviviruses exploit the ER function during infection to gain optimal replication. Multiple independent genome-wide screen studies have identified several ER-associated complexes and individual proteins that are important for flavivirus replication. These complexes such as DNAJC14, Hrd1, EMC, and RTN 3.1A play a crucial role in the construction and function of virus replication and assembly complexes. Thus, these ER- complexes represent promising host targets for developing broad-spectrum anti-flavivirus drugs.

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