Virology 506 (2017) 130–140

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Characterization of virus-specific vesicles assembled by West Nile virus non-structural proteins

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ARTICLE INFO

Keywords:
Flavivirus
Non-structural proteins
Virus-specific vesicles
Replication nanomachine
vRNA synthesis

ABSTRACT

Flavivirus genome encodes seven non-structural proteins (NSPs) and these NSPs are believed to be involved in their genomic RNA replication, of which the mechanism is unclear. We find that West Nile virus (WNV) NSPs were capable of self-assembling membranous vesicles in cells, which are composed of the host endoplasmic reticulum membrane integrated with viral NS1 and NS4A, and possibly NS2A. The vesicles can further organize into replication complex (RC)-associated vesicles which combine both the vesicle and predicted RC components. The authentic RC-associated vesicles were observed in cells transfected with infectious WNV cDNA as well as WNV replicon. Further mutational analysis showed that WNV/DENV heterologous NS polyproteins derived from lethal chimeric recombinants produced abnormal vesicles. Site-directed mutation of either NS2A or NS4A, which resulted in failure of viral RNA replication, caused immature vesicles too. These findings reveal molecular composition and assembly of the virus-specific nanomachine and confirm that these structures are used for the viral RNA replication.

1. Introduction

The flavivirus genus comprises over 70 viruses, many of which are human pathogens (Murphy et al., 1995; Westaway et al., 1986). These include Dengue viruses (DENV), Japanese encephalitis virus, West Nile virus (WNV), Yellow fever virus (YFV), and Zika virus. The latter, historically confined to Africa, re-emerged in South America in 2015 and caused a widespread outbreak of disease in Brazil (WHO, 2016). These viruses share common characteristics, such as: a lipid-enveloped virus particle; a size of 40–65 nm; and a positive-sense, single-stranded RNA of approximately 11,000 bases (Mukhopadhyay et al., 2005). Their genomes contain a long open reading frame (ORF) flanked by 5′ non-coding region (NCR) and by 3' NCR (Lindenbach and Rice, 2003; Brinton, 2014). The ORF translates a polyprotein that is cleaved co- and post-translation to yield three viral structural proteins: capsid, pre-membrane (prM), and envelope (E), and seven nonstructural proteins (NSPs) (Coia et al., 1988; Chambers et al., 1990). Cleavage of the polyprotein is mediated by host cell proteases and by viral NS2B/3 (Falgout and Markoff, 1995; Muller and Young, 2013; Cahour et al., 1992). The viral NS2B/3 protease cleaves at five junction sites within the polyprotein as shown in a diagram (Fig. 1A, a), and to free the C-terminus of the capsid protein from the prM (Cahour et al., 1992; Biedrzycka et al., 1987; Stocks and Lobigs, 1988; Falgout et al., 1991). The cleavage of NS4A C-termini by the NS2B/3 produces an additional 2K fragment, which is required to release signal peptide for NS4A-NS4B cleavage (Lin et al., 1993).

Among the seven NSPs, only NS3 and NS5 are cytoplasmic proteins and found to be replicase complexes containing multiple enzyme functions directly required for viral RNA (vRNA) replication (Lindenbach and Rice, 2003; Brinton, 2014; Mummerer, 2006). The function of other five NSPs in virus production, however, remains to be determined. Research suggests that these NSPs are associated with flavivirus replication complex (RC) (Westaway et al., 1997; Mackenzie et al., 1998). Further studies indicate that YFV NS4A is required for virus infectivity through interaction with NS1, in which an amino acid at NS4A position 42 is essential (Lindenbach and Rice, 1999). The NS4A is also reported to be capable of inducing membrane bend or membrane rearrangement (Miller et al., 2007; Roosendaal al, 2006), suggesting that it may play a role in folding membrane. Whereas, other study demonstrates that DENV NS2A involves in both vRNA synthesis and virion assembly, and the two distinctive processes can be determined by different loci within NS2A gene (Xie et al., 2015).

Flavivirus RNA replication locates in cytoplasm and is membrane-bonded (Salonen et al., 2005; Mackenzie and Westaway, 2001). Earlier

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http://dx.doi.org/10.1016/j.virol.2017.03.016
Received 27 January 2017; Received in revised form 8 March 2017; Accepted 28 March 2017
Available online 04 April 2017

studies have revealed that flavivirus infection induced convoluted membranes and vesicle packets (VP) in cells and that NS1, NS3, and dsRNA localized or associated with the VP (Westaway et al., 1997; JM1 et al., 1996). Recent studies with electron microscope tomography confirmed these organelle-like structures existed in flavivirus infected cells (Welsch et al., 2009; Gillespie et al., 2010; Junjhon et al., 2014).