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Abstract

Impact of Capsid Anchor Length and Sequential Processing on the Assembly and Infectivity of Dengue Virus †

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Abstract: The assembly and secretion of flaviviruses are part of an elegantly regulated process. During maturation, the viral polyprotein undergoes several co- and post-translational cleavage events mediated by both viral and host proteases. Among these, sequential cleavage at the N- and C-termini of the hydrophobic capsid anchor (Ca) at the junction of C-PrM has been considered essential for the production of flaviviruses. Here, using a refined dengue pseudovirus production system, we show that Ca plays a key role in the processing efficiency of dengue virus type 2 (DENV2) structural proteins and the assembly of viral particles. The replacement of the relatively short DENV2 Ca with the homologous regions from West Nile or Zika viruses or, alternatively, the increase in its length, improved cleavage, and hence particle assembly. Furthermore, we show that the substitution of the Ca conserved proline residue (Pro-110), as alanine abolishes pseudovirus production, regardless of the Ca sequence length. Using two experimental approaches, we investigated the need for sequential cleavage (first on the cytosolic side, then on the luminal side) and found that, while cleavage at the Ca-Pr boundary is essential for the assembly of infective particles, the same is not true for cleavage at the C-Ca boundary. We show that both the mature (C) and unprocessed capsids (C-Ca) of DENV2 were equally efficient in packaging the viral RNA and in assembling the infective particles. This was further confirmed with mutants, in which cleavage at the luminal side, by the signal peptidase, occurred independently of cleavage at the cytosolic side, by the viral NS2B/NS3 protease. We thus demonstrate that, unlike other flaviviruses, DENV2 capsid does not require a cleavable Ca sequence and that sequential cleavage is not an obligatory requirement for the morphogenesis of infective particles.

Keywords: capsid anchor; flavivirus; dengue virus; virus assembly; polyprotein processing