Cellular enhancing and restricting factors of dengue virus egress

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KEY MESSAGE

Class II Arfs (Arf4 and Arf5) play a crucial role in the egress of dengue viruses.

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Introduction

Dengue viruses of four serotypes (DV1-4) are the pathogens of dengue fever, dengue haemorrhagic fever, and dengue shock syndrome. An estimated 50 to 100 millions such cases (including 25 000 deaths) occur every year. There is no established treatment for such infections.

The life cycle of dengue viruses, like other enveloped viruses, can be divided into three stages: entry, replication, and egress. Each stage requires participation of the virus itself and/or host cells. For example, viral entry needs a cellular receptor for attachment and penetration through the lipid membrane of the host cell. The hijacking of cellular factors is a common strategy for the virus to complete its life cycle. Although such cellular factors do not assemble into newborn viral particles, they are indispensable for the viral life cycle and thus are potential targets for anti-virus therapy. If these crucial cellular factors cannot be used by the virus, viral replication can be inhibited. Therefore, identifying these crucial factors is important in the fight against the virus.

This study focuses on the late stage of the dengue virus life cycle in host cell—egress. The egress process of enveloped viruses entails assembly followed by transportation of the virus. Assembly of dengue virus occurs in the endoplasmic reticulum (ER). Nascent virions in the ER need to be transported to the Golgi apparatus, and then to the plasma membrane before they are finally released.[1] Cellular trafficking machinery, especially the secretory pathway is believed to be involved in the assembly followed by transportation of dengue virus. We aimed to identify such cellular trafficking factors involved in the egress of dengue virus.

Structurally, dengue virus particles can be divided into internal and external parts.[2] The internal part consists of capsid protein and the RNA genome, which carry viral genetic information and participate in viral replication. The external part is made up of a lipid membrane, prM and E glycoproteins, which are responsible for both viral entry and egress. During viral entry, E glycoprotein binds to cellular receptors and triggers virus-cell membrane fusion. During the secretion process, prM and E glycoproteins interact with a cellular factor along the secretory pathway. For example, the prM protein is cleaved by furin (a major processing enzyme of the secretory pathway) to form M and soluble pr proteins. Such cleavage is critical for maturation of dengue virions. prM and E glycoproteins are also essential for viral assembly occurred in ER. In the presence of capsid protein and RNA genome, prM and E proteins initiate assembly to form nascent virions, whereas in the absence of capsid protein and the RNA genome, prM and E proteins form virus-like particle (VLPs) only.[3]

Dengue VLPs are generated by glycoprotein prME in the absence of the capsid protein and RNA genome. Thus, dengue VLPs consist of the external structure of the dengue virus, but lack the viral genome and thus cannot cause infection (Fig 1). In a previous study, we developed a dengue VLP–producing stable cell line (HeLa-prME) using a codon-optimised dengue prME gene that markedly increases the expression of prME in mammalian cells.[3] The VLPs formed in the ER, and then went through the same egress pathway like dengue virus.[3] Thus, dengue VLPs can mimic the egress of fully formed virus, and therefore constitutes a safe and convenient tool to study the egress of dengue virus.[3]

Methods and results

This study was conducted from August 2008 to July 2010. To identify cellular factors that play important roles in the egress of dengue virus, HeLa-prME cells were used to screen a small interfering RNA (siRNA) library. An siRNA library screen is useful to identify interesting genes from the whole genome of the host cell or from a cluster of genes for specific functions.

Treatment with an siRNA that targets a gene can specifically deplete expression of this gene. Several cellular factors involved in replication of flaviviruses have been identified by screening the whole genome siRNA library. We screened a cellular membrane trafficking siRNA library that targets 122 cellular factors. Each of them was depleted in HeLa-prME cells by siRNA treatment, and their effects on VLPs egress was determined by measuring the amount of VLPs released into the culture medium.

After screening the siRNA library and some other cellular trafficking genes, 23 genes were noted to result in significant reduction of VLPs released, whereas 22 other genes induced a significant increase. Among the cellular factors, Arf 4 and Arf5 were the most interesting. These two factors belong to the ADP-ribosylation factor family, of which six members have been identified so far with only five expressed in humans (Arf2 has been lost). Based on amino acid sequence identity, the six Arfs were grouped into 3 classes: class I (Arf1-3), class II (Arf4, 5), and class III (Arf6).[4]

Simultaneous depletion of class II Arf (Arf4 and Arf5) blocked VLPs for all four dengue serotypes. The crucial role of class II Arfs was confirmed by a rescue experiment using an siRNA-resistant Arf5 gene. By immunofluorescence microscopy, depletion of class II Arfs did not result in VLP accumulation in any compartment downstream of the ER along the secretory pathway. Finally, depletion of class II Arfs resulted in a significant reduction of viral titre for dengue 1 and dengue 4 viruses.

Class II Arfs have been studied much less than other Arfs, and most knowledge on Arf proteins is obtained from Arf1. Arf1 protein is localised in Golgi apparatus and plays an important role in regulating the secretory pathway.[4] The transport from one compartment of the secretory pathway to the next is mediated by the formation of coated membrane vesicles that travel to and fuse with the target organelle. The formation of trafficking vesicles involves membrane curvature, which requires Arf protein. Arf1 protein is recruited to the Golgi membrane and then triggers membrane curvature and vesicle formation and trafficking.[4] Although Arf6 protein functions in different sites, its molecular mechanism is similar.[4] Arf1 protein is hijacked by viruses such as HIV and HCV for assembly or replication, whereas Arf6 protein is hijacked by the coxsackie virus or HIV for virus entry.[4] Thus, class II Arfs are similarly required for the membrane curvature during egress of the dengue virus.[4]

Notably, depletion of Arf1 by siRNA partially reduces the release of dengue VLPs in a manner different from class II Arfs. Arf 4 and Arf5 are functionally redundant during egress of the dengue virus. Our results showed that production of dengue VLPs could not be reduced by Arf4 siRNA or Arf5 siRNA alone, and a single class II Arf protein is sufficient to sustain the proper egress of dengue virus, indicating the overlapping role of Arf4 with Arf5. The functional redundancy of the two Arfs at a site is a common rule for all six Arf proteins.[5] However, such an overlap could not be observed between class II Arfs and Arf1. Simultaneous depletion of Arf1 with any member of class II Arfs did not show stronger inhibition than Arf1 alone. The effect of Arf1 on VLPs release can be explained by Arf1's role in the Golgi apparatus along the secretory pathway. Thus, class II Arfs may be required at a site other than the Golgi apparatus.

There are two types of membrane curvatures during egress of the dengue virus (Fig 2). The first is curvature towards the cytosol to form trafficking vesicles. Class II Arfs were critical for the formation of trafficking vesicle, which brings nascent virions

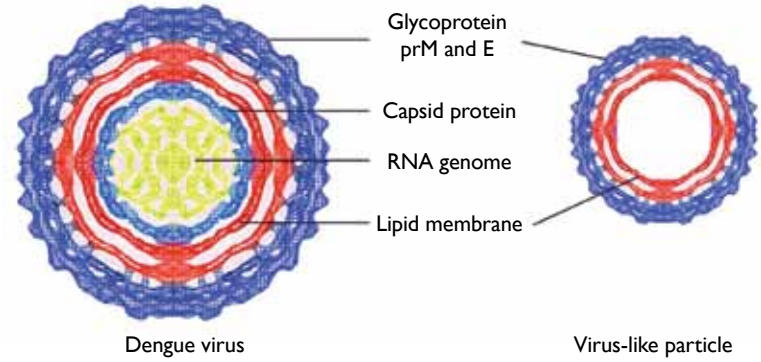

FIG 1. (a) Dengue virus and (b) virus-like particle. Adapted from: Kuhn RJ, Zhang W, Rossmann MG, et al. Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. Cell 2002;108:717-25.

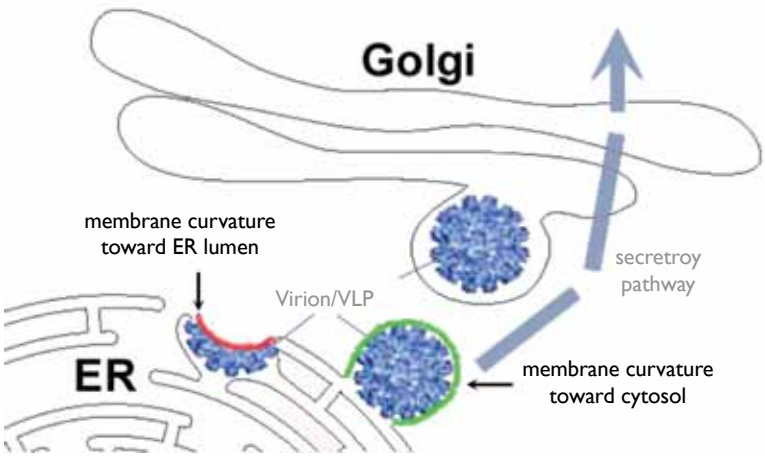

FIG 2. Two types of membrane curvature during the egress of dengue virus: curvature toward the cytosol or the endoplasmic reticulum lumen. Adapted from: Mukhopadhyay S, Kuhn RJ, Rossmann MG. A structural perspective of the flavivirus life cycle. Nat Rev Microbiol 2005;3:13-22.

from ER to Golgi apparatus. The second is curvature towards the ER lumen to form nascent virions. Virus particles or VLPs are spherical membrane structure–like trafficking vesicles and their formation requires the curvature towards the ER lumen. The curved lipid membrane finally becomes a part of the nascent virion (Fig 2). Based on the evidence that class II Arfs (rather than class I Arfs) are partially colocalised with the ER marker calreticulin, and that E protein in HeLa-prME cells is mainly localised to ER, we support the second explanation that class II Arfs are recruited to the ER membrane through interaction with dengue glycoprotein prME and then facilitate membrane curvature and formation of the dengue particle.

Although the mechanism by which class II Arfs are involved remains unknown, our findings shed new light on a molecular mechanism used by dengue viruses during the late stages of their replication cycle and demonstrate a novel role for class II Arf proteins.

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References

1. Mukhopadhyay S, Kuhn RJ, Rossmann MG. A structural perspective of the flavivirus life cycle. Nat Rev Microbiol 2005;3:13-22.
2. Kuhn RJ, Zhang W, Rossmann MG, et al. Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. Cell 2002;108:717-25.
3. Wang PG, Kudelko M, Lo J, et al. Efficient assembly and secretion of recombinant subviral particles of the four dengue serotypes using native prM and E proteins. PLoS One 2009;4:e8325.
4. D'Souza-Schorey C, Chavrier P. ARF proteins: roles in membrane traffic and beyond. Nat Rev Mol Cell Biol 2006;7:347-58.
5. Volpicelli-Daley LA, Li Y, Zhang CJ, Kahn RA. Isoform-selective effects of the depletion of ADP-ribosylation factors 1-5 on membrane traffic. Mol Biol Cell 2005;16:4495-508.