VIRAL RNA DELIVERY BY RECOMBINANT DENGUE VIRUS CAPSID PROTEIN: VIRAL RNA REPLICATION, VIRION ASSEMBLY AND THEIR BIOLOGICAL IMPLICATIONS

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For all enveloped viruses, genome access to the cytoplasm is dependent on viral envelope fusion with a cellular membrane, which is triggered by a viral fusion protein. In the case of dengue virus (DENV), it is currently known that genome release into the cytoplasm occurs through envelope (E) protein-mediated membrane fusion. However, based on recent results, we hypothesize that DENV capsid (C) protein may cooperate with E protein during fusion stages. Our group recently characterized DENV C protein as a supercharged protein, which is able to penetrate cells delivering functional cargoes. In this work, we demonstrate that DENV2 C protein was able to internalize DENV1 RNA (derived from DENV1 infectious clone vBACDV1) in hepatic and insect cells. Lipofectin-mediated transfection of DENV1 RNA was used as a positive control. After DENV2 C protein- and lipofectin-mediated transfection, DENV1 RNA expression and translation was monitored through qPCR, immunofluorescence microscopy and flow citometry studies using DENV specific primers and antibodies against DENV E protein, respectively. Our results show successful viral protein expression within 96 hours post DENV2 C protein-mediated transfection. Also, transfection of DENV1 RNA with DENV2 C protein promotes virus assembly, since infectious DENV particles were detected in the medium within 96 hours post transfection. Although, percentage of transfected cells expressing DENV E protein is higher in lipofectin-mediated transfection, DENV2 C protein-mediated transfection promoted efficient virus replication and assembly, since released DENV particles levels are comparable to lipofectin positive control. Curiously, DENV2 C protein-internalized RNA early replication kinetics was different from that of lipofectin-delivered RNA. Taken together, these results not only reinforce the role of C protein in viral genome release into the cytoplasm, but also suggest a role for DENV C protein in viral RNA addressing and replication.

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