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Kinetics of the association of dengue virus capsid protein with the granular component of nucleolus

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ABSTRACT

Dengue virus (DENV) replicates in the cytoplasm but translocation of the capsid protein (C) to the nucleoli of infected cells has been shown to facilitate virus multiplication for DENV-2. This study demonstrates that the nucleolar localization of C occurs with all four serotypes of DENV. The interaction of C with the nucleolus was found to be dynamic with a mobile fraction of 66% by FRAP. That the C shuttled between the nucleus and cytoplasm was suggested by FLIP and translation inhibition experiments. Colocalization with B23 indicated that DENV C targeted the granular component (GC) of the nucleolus. Presence of DENV C in the nucleolus affected the recovery kinetics of B23 in infected and transfected cells. Sub-nucleolar localization of DENV C of all serotypes to the GC, its mobility in and out of the nucleolus and its affect on the dynamics of B23 is being shown for the first time.

1. Introduction

DENV, which belongs to family Flaviviridae, genus *Flavivirus,* has a positive sense ssRNA genome of ~11 kb. DENV replication takes place in the cytoplasm (Bulich and Aaskov, 1992), however the C is known to translocate to the nucleoli (Wang et al., 2002; Iglesias et al., 2015). The C is a small (~12 kDa), highly basic protein with three nuclear localization signal (NLS) (Sangiambut et al., 2008). The movement of the C to the nucleus is an active process and mediated by its interaction with alpha-importin (Bhuvanakantham et al., 2009). Different nuclear proteins have been shown to interact with DENV C, consequential to virus multiplication as well as cellular functions. The interaction of C with DAXX was shown to sensitize hepatic cells to Fas-mediated apoptosis (Limjindaporn et al., 2007). Nucleosome assembly was shown to be inhibited by the formation of heterodimers of C with histones (Colpitts et al., 2011). The C was shown to interact with nuclear protein hnRNP K (Chang et al., 2001). Subsequently, it was shown that DENV-2 infection resulted in the sequestration of hnRNP K from nucleus to cytoplasm. Silencing of hnRNP K inhibited virus multiplication (Brunetti et al., 2015). Interaction of C with nucleolin in the nucleolus was shown to be important for formation of stable and functional nucleocapsid, disruption of the interaction resulted in reduced infectivity of virions (Balinsky et al., 2013). Nucleolus is the ribosome factory of the cell and has three functionally different compartments the fibrillar center (FC), the dense fibrillar component (DFC) and the granular component (GC) (Boisvert et al., 2007; Hernandez-Verdun, et al., 2010). This study was undertaken to determine which functional compartment of the nucleolus was targeted by DENV C and the dynamics of the interaction.

2. Results

2.1. Nucleolar localization of DENV C

Presence of DENV C in the nucleolus was reported previously for DENV-4 and 2 (Tadano et al., 1989; Bulich and Aaskov, 1992). To determine whether the accumulation of C in the nucleolus was a common feature for all four serotypes, BHK-21 cells were infected with DENV-1, 2, 3 and 4. The C was detected by IFA using C-specific monoclonal antibody (C-MAb). The mean fluorescence intensities (MFI) was measured for 10 cells each. The C was present in the nucleolus (MFI of 228) of cells infected with all four DENV serotypes. The C was also observed in the cytoplasm (MFI of 198) and at lower levels (MFI of 100) in the nucleoplasm (Fig. 1).

2.2. Sub-nucleolar localization of DENV C

The nucleolar localization of C was dissected further by live cell imaging of cells transfected with C gene cloned into mammalian expression vectors. In cells transfected with pVax-C (Fig. 2A) and pGFP-C (Fig. 2B), fluorescence was stronger in the nucleolus than in the cytoplasm compared to the infected cells. Tagging the gene with

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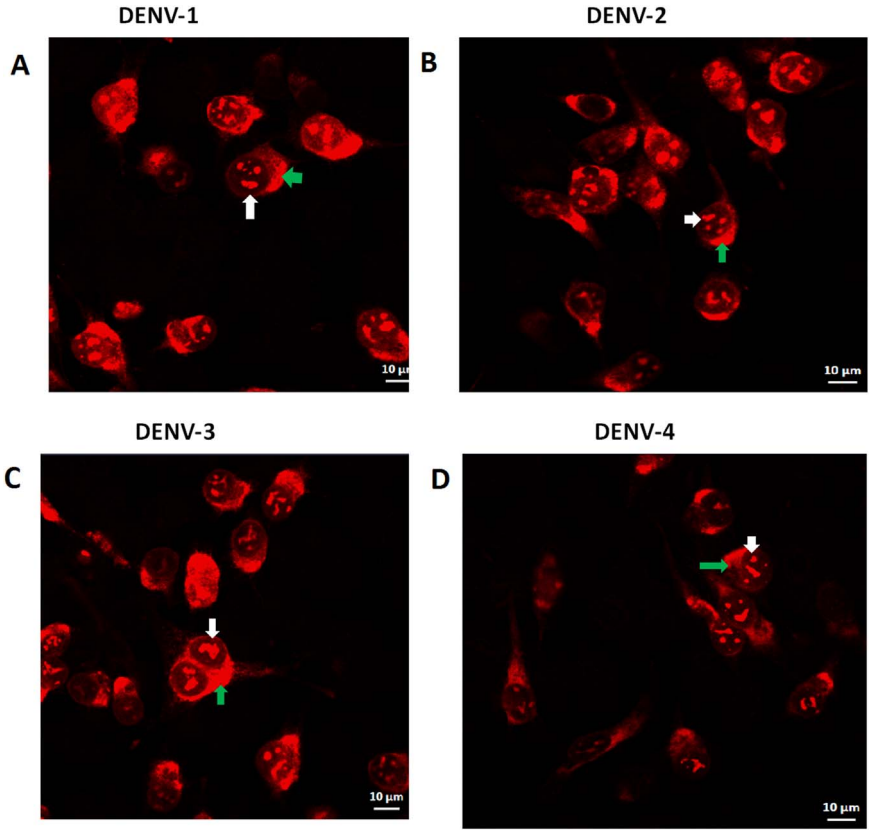

Fig. 1. Nucleolar localization of DENV C is common for all four serotypes. BHK-21 cells infected with different serotypes (A) DENV-1 (B) DENV-2 (C) DENV-3 and (D) DENV-4 were fixed 48 h after infection, permeabilized and stained with C-MAb followed by anti-mouse-TRITC. White arrow shows nucleolar and green arrow shows cytoplasmic accumulation of the C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

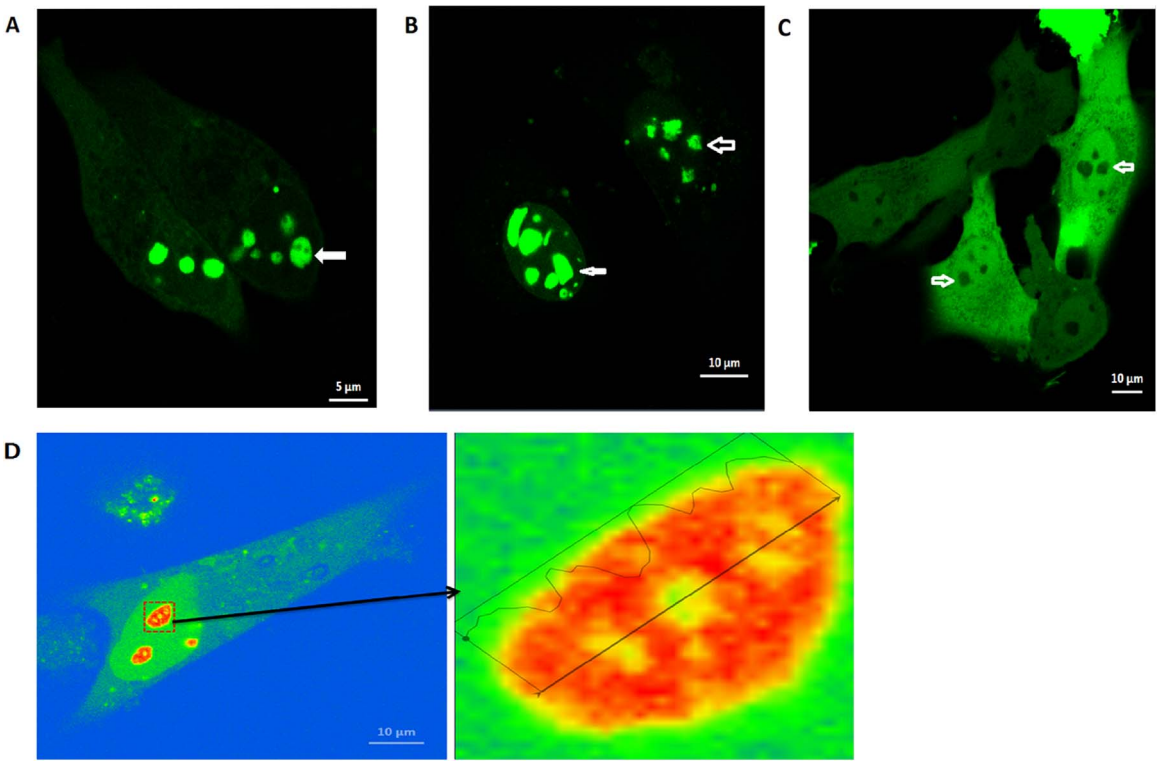

Fig. 2. Localization of DENV C in the nucleolus of BHK-21 cells. Cells transfected with (A) pVax-C, fixed and stained with C-MAb followed by anti-mouse-FITC. Live cell image of cells transfected with (B) pGFP-C and (C) pGFP. The arrows point to the nucleoli. (D) Pseudocolored image showing heterogeneous distribution of DENV C in the nucleolus of pGFP-C transfected cells, gradient from blue to red indicates low to high concentration of the protein in the cell. Cropped image showing the MFI profile across the single nucleolus. Images are representative of majority of cells observed in three experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)