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Strand-like structures and the nonstructural proteins 5, 3 and 1 are present in the nucleus of mosquito cells infected with dengue virus

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ABSTRACT

Dengue virus (DENV) is an arbovirus, which replicates in the endoplasmic reticulum. Although replicative cycle takes place in the cytoplasm, some viral proteins such as NS5 and C are translocated to the nucleus during infection in mosquitoes and mammalian cells. To localized viral proteins in DENV-infected C6/36 cells, an immunofluorescence (IF) and immunoelectron microscopy (IEM) analysis were performed. Our results indicated that C, NS1, NS3 and NS5 proteins were found in the nucleus of DENV-infected C6/36 cells. Additionally, complex structures named strand-like structures (Ss) were observed in the nucleus of infected cells. Interestingly, the NS5 protein was located in these structures. Ss were absent in mock-infected cells, suggesting that DENV induces their formation in the nucleus of infected mosquito cells.

1. Introduction

The four distinct dengue virus (DENV 1–4) serotypes belong to the family *Flaviviridae*, genus *Flavivirus* and are responsible for 390 million of infections each year according to World Health Organization (WHO). DENV is transmitted by *Aedes* mosquitoes mainly *Aedes aegypti* and *Aedes albopictus* in the tropical and subtropical regions of the world (Back and Lundkvist, 2013; Bhatt et al., 2013; Guzman and Harris, 2015). DENV genome consists of a positive-sense RNA of ~11 Kb that during infection is translated into a polyprotein, which is cleaved by cellular and viral proteases into the structural proteins C, prM, and E, and the seven nonstructural (NS) proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 (Chambers et al., 1990; Lindenbach and Rice, 2003). The nonstructural proteins are involved in viral replication and assembly of the new viral progeny in the endoplasmic reticulum (ER) of infected cells. Although it has been reported that DENV has a cytoplasmic replicative cycle, some viral proteins such as the structural capside protein (C) (Bulich and Aaskov, 1992; Makino et al., 1989; Netsawang et al., 2010; Sangiambut et al., 2008; Tadano et al., 1989; Tiwary and Cecilia, 2017; Wang et al., 2002), and the nonstructural protein 5 (NS5) (Hannemann et al., 2013; Kapoor et al., 1995; Kumar et al., 2013; Miller et al., 2006; Pryor et al., 2007; Tay et al., 2016) are relocated to the nucleus during infection in mammalian cells. Although the functions of C and NS5 in the nucleus are unclear, it could be related with the control of gene expression of the infected cells (IL-8 expression) (Medin et al., 2005). In this regard, in a recent report, the interaction of NS5 with components of the U5 snRNP was confirmed in mammalian cells. This interaction reduces the efficiency of pre-mRNA splicing and renders a favorable cellular environment for DENV replication (De Maio et al., 2016).

In contrast to mammalian cells, little is known about the localization and function of viral proteins in the nucleus of mosquito cells, however, the presence of C and NS5 proteins in the nucleus has been previously described in infected C6/36 cells (Bulich and Aaskov, 1992; Hannemann et al., 2013; Junjhon et al., 2014; Sangiambut et al., 2008; Tadano et al., 1989). In this study, we perform an immunofluorescence (IF) and immunoelectron microscopy (IEM) analysis to determine which viral proteins are present in the nucleus of DENV-2 infected C6/36 cells.

2. Materials and methods

2.1. Cell culture and viral strain

C6/36 cells, adapted to grow at 35 °C (Igarashi, 1978; Kuno and Oliver, 1989), were grown in a CO_2-free incubator (Lab Line) and were cultured (Corning) in Minimal Essential Medium (MEM) (Invitrogen) supplemented with 10% fetal bovine serum (Sigma), vitamins

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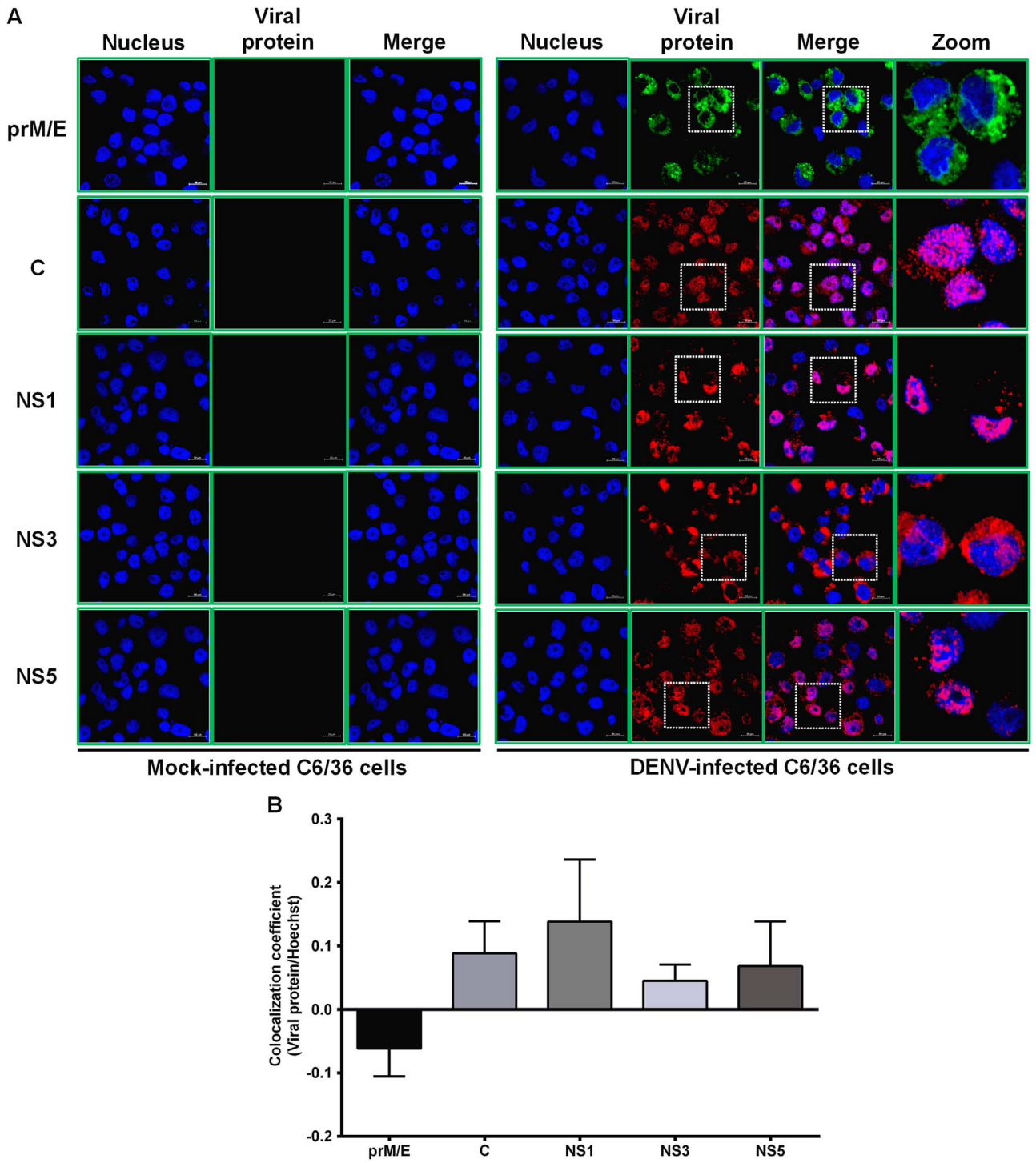

Fig. 1. Immunolocalization of viral proteins in DENV-infected C6/36 cells by immunofluorescence confocal microscopy. (A) C6/36 cells were mock-infected or infected with DENV-2, fixed, and immunolabeled with specific antibodies anti-prM/E, -C, -NS1, -NS3, or -NS5 viral proteins. Nuclei were stained with Hoechst (blue). (B) The graph compares the colocalization coefficient mean ± SD, through Pearson's correlation coefficient, among Hoechst (blue) and viral proteins (green and red).

(Invitrogen), 0.034% sodium bicarbonate (J.T. Baker), 100 μg/mL streptomycin, and 100 U/mL penicillin (Sigma).

DENV-2 (New Guinea C strain) (donated by InDRE, Instituto Nacional de Diagnóstico y Referencia Epidemiológica, Mexico) was propagated in BALB/c suckling mice brains (Gould and Clegg, 1991) and viral titer was determined by plaque assay in BHK-21 cells as was previously reported (Juárez-Martínez et al., 2012). BALB/c suckling mice brains from uninfected or mock-infected mice were used as a control.

2.2. DENV infection

C6/36 cells, seeded at 80% confluence, and incubated at 35 °C during 24 h were washed three times with Hanks medium, and mock-infected or infected with DENV serotype 2 at a MOI of 5 for 2 h at 35 °C; then, cells were washed with acid glycine (pH 3) to inactivate non-internalized virus, washed 3 times with PBS and the infection was allowed to proceed for 48 h at 35 °C.