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*Abstract*

# Zika Virus sfRNA Plays an Essential Role in the Infection of Insects and Mammals †

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† Presented at Viruses 2020—Novel Concepts in Virology, Bacelona, Spain, 5–7 February 2020.

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Published: 1 July 2020

**Abstract:** Similar to other flaviviruses, Zika virus (ZIKV) produces abundant subgenomic flavivirus RNA (sfRNA) derived from the 3′ untranslated region. The molecular mechanisms that determine the functions of sfRNA are currently not completely understood. Here, we created ZIKV mutants deficient in sfRNA production and employed them to investigate the role of this RNA in virus interactions with mammalian and insect hosts. We found that in mosquitoes, sfRNA facilitates virus replication and is required for ZIKV dissemination into saliva and virus transmission. The production of sfRNA was found to have no effect on the RNAi pathway, but instead downregulated the expression of genes involved in the regulation of apoptosis. The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) of histological sections from infected mosquitoes confirmed that sfRNA prevents the apoptotic death of infected cells, thus identifying inhibition of apoptosis as a novel mechanism of sfRNA action in mosquitoes. We also found that sfRNA facilitates ZIKV replication in mammalian cells, mice, and human brain organoids. Moreover, ZIKV mutants deficient in sfRNA production were unable to form plaques, cause the death of human brain organoids, or establish infection in the mouse foetal brain. We then found that the proviral activity of sfRNA in mammalian cells relies on its ability to suppress type I interferon signalling. We showed that this is achieved via the inhibition of phosphorylation and the nuclear translocation of STAT1. In addition, we found that the production of sfRNA in the ZIKV infection of human brain organoids is associated with the suppression of multiple genes involved in brain development, indicating that sfRNA can be involved in the disruption of brain development associated with ZIKV infection.

**Keywords:** Zika virus; subgenomic flavivirus RNA; apoptosis; type I interferon; foetal brain infection