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A Replicon-Baculovirus Model for Efficient Packaging of Hepatitis E Virus RNA and Production of Infectious Virions

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Abstract : Hepatitis E virus (HEV) is an emerging RNA virus that causes acute and chronic liver disease with a global mortality rate of about 2%. Despite milestone developments in understanding of HEV biology, there is still lack of a robust culture system or animal model. Therefore, in a novel approach, two recombinant-baculoviruses (vBac-ORF2 and vBac-ORF3) that could overexpress HEV ORF2 (structural/capsid) and ORF3 (nonstructural/regulatory) proteins, respectively were constructed. The established HEV-SAR55 (genotype 1) replicon that contained GFP gene, in place of ORF2/ORF3 sequences was in vitro transcribed, and GFP production in RNA transfected S10-3 cells was scored by FACS. Enhanced infectivity, if any, of nascent virions produced by exogenously-supplied ORF2 and viral RNA by co-expression of ORF3 was tested on naïve HepG2 cells. Co-transduction with vBac-ORF2/vBac-ORF3 (108 pfu/microL) produced high amounts of native ORF2/ORF3 in approximately 60% of S10-3 cells, determined by immunofluorescence microscopy and Western analysis. FACS analysis showed about 9% GFP positivity of S10-3 cells on day6 post-transfection (i.e, day5 post-transduction). Further, FACS scoring indicated that lysates from S10-3 cultures receiving the RNA plus vBac-ORF2 were capable of producing HEV particles with about 4% infectivity in HepG2 cells. However, lysates of cultures co-transduced with vBac-ORF3, were found to further enhance virion infectivity by approximately 17%. This supported a previously proposed role of ORF3 as a minor-structural protein in HEV virion assembly and infectivity. In conclusion, the present model for efficient genomic RNA packaging and production of infectious virions could be a valuable tool to study various aspects of HEV molecular biology, in vitro.

Keywords : chronic liver disease, hepatitis E virus, ORF2, ORF3, replicon
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