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Baculovirus vector for expression in mammalian cells and its application for study on biological functions of hepatitis C virus core protein

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The baculovirus, *Autographa californica* nuclear polyhedrosis virus (AcNPV), expression system has been used for the expression of a wide variety of foreign genes because of its high level-expression in insect cells. Recently, it was shown that AcNPV can infect human hepatocytes and reporter gene was highly expressed from CMV and RSV promoters. We also constructed recombinant baculoviruses carrying reporter genes under the control of CAG promoter which exhibits stronger expression than the CMV and RSV promoters. Expression of the reporter gene was observed not only in hepatocytes but also in most of the cell lines tested. Then, we compared the efficiencies of gene expression of baculovirus vector with replication deficient adenovirus vector by using the identical expression unit in various cell lines. Although reporter gene expression was higher in most of the cell lines infected with the adenovirus, in HepG2 and pig kidney cell lines almost the same level of expression was observed by both vectors.

Hepatitis C virus (HCV) is a major causative agent of post transfusion and sporadic non-A, non-B hepatitis. The core protein of HCV is expected to bind with viral sense RNA to form a nucleocapsid because of its high content of basic amino acid residues. However, there is no report yet demonstrating a specific interaction of the core protein with the genomic RNA. To clarify the binding property of HCV core protein with viral genomic RNA, we synthesized various regions of viral and anti-viral sense RNA of HCV *in vitro* and transfected into HepG2 cells expressing HCV core protein. Specific binding of core protein was observed with a full length-positive sense-HCV RNA, but not with negative sense. In addition to functioning in the formation of nucleocapsids of HCV, data have been accumulating to suggest that core protein has versatile functions as regulatory proteins. HepG2 cells expressing HCV core protein showed apoptotic changes in response to stimulation with anti-Fas monoclonal antibody. Furthermore, formation of cytoplasmic vacuoles containing apolipoprotein was observed not only in the cell lines but also in transgenic mice.

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