F037

Partial Reconstitution of Hepatitis C virus RNA Polymerization by Heterologous Expression of NS5B Polymerase and Template RNA in Bacterial Cell

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The hepatitis C virus is a major etiological agent causing chronic hepatitis in humans The current study presents a surrogate model system using a viral polymerase and RNA template. A plasmid expressing the HCV NS5B polymerase was maintained with a plasmid containing a reporter gene in an E. coli cell The reporter construct contained the HCV 5' UTR followed by a luciferase gene with a specific orientation so that a minus-sense transcript containing the luciferase fused to the 5' UTR was produced after the initial transcription When the HCV NS5B polymerase was expressed in the same cell, the primary transcript was recognized by the polymerase due to the presence of the minus-sense 5' UTR, and a secondary transcript containing a plus-sense luciferase gene produced Thus, a simple luciferase assay was able to measure the HCV NS5B polymerase activity. The production of minus and plus-sense transcripts was confirmed by an RT-PCR, while the production of HCV NS5B and expression of the reporter luciferase in the bacterial cell were confirmed by immunofluorescence microscopy The polymerization occurred in the absence of any other viral/host factors

F038

The Lethal Phenotypes of Dna2 mutations Are Rescued by Overexpression of the DNA Repair Proteins in *Saccharomyces cerevisiae*

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Dna2 protein involved in Okazaki fragment processing is a multifunctional enzyme with two essential enzyme activities, endonuclease and helicase. The endonuclease activity is essential to remove RNA-DNA primers of Okazaki fragments, while the helicase facilitates removal of secondary structures formed in the primers In an effort to identify genes interacting genetically with DNA2, multi-copy suppressors were screened in yeast that rescue the lethality of Dna2 mutants devoid of helicase (dna2KE) or endonuclease (dna2DA) activities A putative helicase, MPH1, was identified as a suppressor for dna2KE. Purified Mph1 protein possessed both ATPase and helicase activities, suggesting that it might replace the function of Dna2 helicase The suppressors for dna2DA mutant encode the nucleases involved in the nucleotide excision or double-strand break repair pathways They also contain several novel proteins that associate with these enzymes These results suggest that some DNA repair proteins may participate in removing the unprocessed RNA-DNA primers which are not cleaved by Dna2 and/or other Okazaki fragment processing enzymes

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F039

A Novel Interferon Resistance Mechanism of Hepatitis C Virus (HCV) Mediated by NS5B Protein

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Two mechanisms of interferon resistance for hepatitis C virus are known One is mediated by E2 protein and the other is mediated by NS5A protein Both target host PKR for their action We report here a third mechanism mediated by NS5B protein An interferon effector molecule called p56 was recently identified. It binds to p48 subunit of eIF3 complex to inhibit translation. We showed that NS5B also bound to p48 of eIF3 It was demonstrated by yeast two hybrid analysis GST-pulldown assay. The subcellular colocalization was confirmed by immunofluorescence confocal microscopy. The inhibitory effect of interferon alpha was diminished by the presnece of NS5B in a reproter assay from cultured human hepatocyte. When NS5B was present, the inhibitory effect of p56 on translation was diminished in an in vitro lysate assay The inhibition was in a dose-dependent manner

F040

Identification of Genes Regulated by *luxO* Homologue in *Vibrio vulnificus* MO6 24/O Using a Small-scale DNA Microarray

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Vibrio vulnificus is an opportunistic pathogen, which causes a primary septicemia on human As in other *Vibrio* species, *V vulnificus* contains *luxO* homologue, which is known to regulate the expression of genes related with the luminescence and the virulence in *V harveyi* and *V cholerae*, respectively To identify the genes regulated by LuxO in *V vulnificus*, a small-scale DNA microarray was constructed, which comprises 160 PCR products, putatively encode functions associated with pathogenicity and gene regulations, derived from the genome of *V vulnificus* The cDNAs of wild-type and *luxO* null-mutant were hybridized on SGVV v 1 0 1 chip which was printed with 640 spots (quadruplicates of 160 genes) We carried out two sets of hybridizations and another dye-swap of one set of hybridizations To analyze the array profile, we employed two statistical procedures, the SAM procedure and the Lonnstedt and Speed's empirical Bayes procedure using B statistic As a result, 4 differentially expressed genes of statistical significance, including DNA helicase and DNA binding protein H-NS were identified

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