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CHARACTERIZATION OF RECOMBINANT HEPATITIS C VIRUS NS5B GENE PRODUCT (RDRP).

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Chronic infection with hepatitis C virus (HCV) represents a major public health problem affecting approximately 4.9 million people in the USA. The HCV polymerase is a possible target for molecular-based therapeutics. The NS5B gene contains a sequence motif conserved in viral RNA-dependent RNA polymerases (RDRP). We have subcloned the NS5B gene into prokaryotic expression vectors and expressed a protein in *E. coli* that encodes the HCV RDRP. We have solubilized recombinant RDRP protein under nondenaturing conditions and partially purified the enzyme. Sera from patients with chronic hepatitis C react with the recombinant RDRP during Western blotting. The recombinant protein has RNA-dependent RNA polymerase activity assays using poly(A) or globin $_m$RNA as a template. RNA products were analyzed using agarose/formaldehyde gels and our results demonstrate that the enzyme synthesizes template-sized RNA products in a primer-dependent manner. The enzyme is most active at 30°C, requires Mg^{++} and shows little 3' terminal transferase activity. Conclusion: Enzymatically active recombinant HCV RDRP has been expressed in *E. coli*. HCV RDRP has characteristics similar to other primer-dependent viral RDRPs. This recombinant polymerase should permit detailed studies of the mechanism of HCV genome replication and aid the identification of small molecule inhibitors.

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Characterization of A Novel Fusion Inhibitor of Influenza A Virus.

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A novel fusion inhibitor of influenza virus has been identified and characterized. The inhibitor, BMY-27709, has an IC_{50} of about 1 μM in a plaque reduction assay and 3-8 μM in a multicycle growth assay for influenza A/WSN/33 (H1N1) virus in MDBK cells. The inhibitory activity of BMY-27709 is hemagglutinin(HA)-specific. It is active against both H1 and H2 subtype viruses, but inactive against H3 subtype viruses as well as influenza B/Lee/40 virus. The HA protein was confirmed to be the target of BMY-27709 through the use of HA reassortant virus and isolation of drug resistant viruses. All drug resistant viruses contain amino acid mutations in the HA. Most of these mutations map to a similar region near the fusion peptide of the HA2 subunit. Mechanism of action studies show that inhibition is mediated through specific interaction with the HA protein. This interaction prevents the native HA trimmer from undergoing the low-pH induced conformational change, and thereby inhibits HA-mediated fusion between viral and cellular membranes.