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Determination of Functional Domains In The Influenza Virus Polymerase Through Site-directed Mutagenesis and Transient Expression

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The ribonucleoprotein (RNP) complex of influenza virus consists of equimolar amounts of three polymerase proteins (PB2, PB1, and PA), as well as viral RNA encapsidated by the nucleoprotein (NP). Recently, we had shown that these four viral proteins are sufficient for transcription and replication of synthetic RNP *in vivo*. This was determined through the co-expression of these four proteins using vaccinia virus vectors (Huang *et al*. J. Virol. 64, 5669-5673, 1990).

In this report, this system has been extended in several ways. First, expression of viral polymerase proteins was accomplished directly from transfected plasmid DNA under the control of T7 RNA polymerase, making site-specific mutagenesis feasible. Second, together with the initial *in vivo* replication/transcription assay which utilizes the expression of a reporter gene (CAT), several *in vitro* assays associated with discrete polymerase functions were developed. These activities include complex formation, viral endonuclease cleavage, cap-primed transcription, and viral RNA binding. Thus, rationally designed mutations in the viral polymerase proteins can be made and their defects defined with these assays. In this way, precise functions and functional domains of the various polymerase proteins can be resolved.

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Antisense Inhibition of Respiratory Syncytial Virus Replication

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We have evaluated oligodeoxyribonucleotides as antisense inhibitors of respiratory syncytial virus replication. HEp-2 cells were infected with RSV strain A2 (moi=0.01-0.1) and incubated for 3-5 days. Virus replication was measured by plaque assay, ELISA, or virus yield assay. Antisense phosphorothioate oligodeoxynucleotides were targeted to messenger RNAs and viral RNA. mRNA-targeted oligos were designed to disrupt translation of individual viral gene products. vRNA-targeted oligos were designed to interfere with transcription by the virally encoded RNA dependent RNA polymerase. Using ELISA, EC_{50} values measured for mRNA-targeted oligonucleotides were ≥10μM. Antisense oligonucleotides targeted against the vRNA were more potent inhibitors (EC_{50} values ≈1 μM). Under the same experimental conditions, ribavirin inhibited RSV with an EC_{50}≈10 μM. Oligonucleotides did not inhibit cell growth (as measured by XTT assay) at concentrations as high as 100 μM. These results indicate that antisense oligonucleotides targeted against RSV viral RNA can effectively inhibit RSV replication.