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Cloning and Expression of the Herpes Simplex Virus Type 1 DNA Polymerase in Bacteria. David I. Dorsky and Clyde S. Crumpacker. Division of Infectious Diseases, Beth Israel Hospital and Harvard Medical School, Boston, MA, USA.

The HSV pol gene was mobilized from a vector which could express a functional gene product downstream from the SV40 early promoter in COS-1 cells.[1] The pol gene was then inserted into the T7-based expression vector T7-7 as an in-frame fusion at the pol SnaBI site (deleting the amino-terminal 67 residues), downstream from the phage T7 gene 10 initiator, fused at the fourth codon. The resulting construction was transformed into E. coli containing pGP1-2, expressing the T7 RNA polymerase from a lambda phage P_L promoter controlled by a heat-sensitive lambda repressor.[2] When cells containing both plasmids are induced at 42° C., a protein of 140 kd is induced and the expression is rifampin-resistant. The pol fusion protein accumulates to form a visible band on Coomassie blue-stained SDS-PAGE gels, but remains in the insoluble fraction of lysates prepared from induced cells. Efforts are underway to renature this insoluble fusion protein to study its enzymatic properties. The fusion protein is also being used as an immunogen to prepare pol-specific antibodies in rabbits.

1. Dorsky and Crumpacker, J. Virol. 61:1704-1707 (1987).
2. Tabor and Richardson, Proc. Nat. Acad. Sci. 82:1074-1078 (1985).

I-5

Inhibition of Calf Thymus DNA Topoisomerase I -Mediated Relaxation of Supercoiled DNA By 2',5'-Oligoadenylates

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DNA topoisomerases interconvert various topological isomers of DNA and play key roles in replication and gene expression. In addition, virus- or host-encoded topoisomerases may be required for the replication of some viruses. The possible involvement of the interferon-induced 2-5A system in cell growth, regulation, and cell differentiation has led us to investigate the effects of 2',5'-oligoadenylates on mammalian topoisomerases. We have found that the calf thymus type I topoisomerase is inhibited by a variety of 2-5A congeners. The extent of inhibition is dependent upon the number of residues and the degree of phosphorylation at the 5'-terminus. For instance, the Ki for p5'A2'p5'A is almost twice the Ki for the triphosphate ppp5'A2'p5'A and the Ki for ppp5'A2'p5'-A2'p5'A is seven-fold lower than that for A2'p5'A2'p5'A. The hexamer 2',5'-p(Ap)$_5$A (Ki=2.5 μM) is about 600-fold more potent than the dimer p5'A2'p5'A. These results suggest the possibility that host and viral topoisomerases may represent another target of 2'5'-oligoadenylates.