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CHARACTERIZATION OF THE MAMMALIAN DNA POLYMERASE GENE AND PROTEIN

Annual Progress Report

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Progress Report **Attachment #1**

Characterization of the mammalian DNA polymerase gene(s) and enzyme(s)

Abstract

Two Genes for DNA polymerase delta were identified from the wild type Chinese hamster ovary cells. These genes were cloned via RT-PCR from mRNA prepared the chinese hamster ovary cells using primers specific to conserved sequences of the DNA polymerase δ gene. The first gene encodes a PCNA dependent DNA polymerase δ gene whereas the second gene encodes a PCNA independent DNA polymerase δ gene. Methods were developed to clone these genes in expression vector and host systems. The role of the two genes in DNA replication and repair was determined.

Introduction

Consistent with our long term goals to understand the mechanism of eukaryotic nuclear DNA replication we have successfully cloned DNA polymerase delta gene(s) from the chinese hamster ovary cells. The DNA polymerase δ gene was chosen because of its smaller size and the fact that it is involved in the synthesis of both leading and lagging strands of DNA during the process of DNA replication.

Results

1. Molecular cloning and characterization of the PCNA dependent catalytic subunit of DNA polymerase δ gene of the chinese hamster ovary cells.

The gene encoding the catalytic subunit of DNA polymerase delta (pol δ) in Chinese hamster ovary (CHO) cells was cloned from cDNA library. it is 3389 base pair long, encoding a polypeptide of 1103 amino acids with a calculated molecular weight of 123,465. The CHO pol δ catalytic subunit was found to be 96% homologous in amino acid sequence to that of mouse, 90% to those of bovine and human, and 50-55% to those of *S. cerevisiae* and *S. pombe*. A comparison with the published DNA polymerase sequences showed that the CHO pol δ catalytic subunit had all of the conserved regions for YGDTD-type DNA polymerase and the conserved regions found in other pol δ's. The gene for the catalytic subunit of CHO pol δ was stably established under a T7 promoter in bacteria and was successfully expressed with polymerase delta activities.

2. Molecular cloning and Expression of the Catalytic Subunit of DNA Polymerase Delta from Chinese Hamster Ovary Cells

DNA pol δ and PCNA are required for both DNA replication[1,2] and nucleotide excision repair[3-5]. However, while p21 inhibits DNA replication upon DNA damage by binding to PCNA, it has no effect on repair[6-8], suggesting other polymerase activities may be involved in DNA repair. In addition, two different forms of δ have been purified from several organisms[9-15]. Here we report the cloning and the expression of a novel pol δ (pol δ') from CHO cells. When compared to the sequence of pol δ, pol δ' lacked Gly-63 and has a Pro-to-Ser change in the conserved region IV. The activities of pol δ resembled those of pol δ, but was PCNA-independent. The expression of pol δ' was constitutive through the cell-cycle, but was significantly lower than that of pol δ in proliferating cells and increased significantly after UV-irradiation. Pol δ', like pol δ, may be involved in DNA repair synthesis.

Publications: Following publications are the result of the current DOE grant.

1. Feher, Z. and N.C. Mishra 1994.
Aphidicolin-resistant chinese hamster ovary cells possess altered DNA polymerases of the α-family.
Biochim Biophys. Acta 1218 35-47

2. Feher Z. and N.C. Mishra. 1995.
An aphidicolin-resistant mutant of chinese hamster ovary cell with altered DNA polymerase and 3' exonuclease activities.
Biochim Biophys Acta (in press)

3. Singleton, Robert 1994 Ph.D. dissertation, The University of South Carolina.

4. Singleton, R. and N.C. Mishra. 1995.
Genetic evidence that aphidicolin inhibits *in vivo* DNA synthesis in Chinese hamster ovary cells.
Mol. Gen. Genetics 247: 462-470

5. Liu, Zitong and N.C. Mishra 1995
Single tube method for plasmid mini prep form large numbers of clones for direct screening by size or restriction digestion.
Biotechnique 18: 214-215

6. Mishra N.C. 1995
The Molecular Biology of Nuclease 1-304 pages. CRC Press, Boca Raton, Florida.

7. Liu, Zitong and N.C. Mishra 1995.
Molecular cloning and expression of the catalytic subunit of DNA polymerase Delta from Chinese hamster ovary cells.
Journal Biol. Chem. (submitted)

8. Liu, Zitong and N.C. Mishra 1995.
Molecular cloning expression and characterization of a novel PCNA-independent DNA polymerase Delta from Chinese hamster ovary cells.
Nature (submitted)

Invited Presentation and Future Publications:

In addition to Zitong Liu, Peter Lauzon is also working on the project supported by the DOE. Dr. Z. Feher, a post doctoral fellow, who worked on the projected has returned to Medical University in Debrecen, Hungary where he is a now an Associate Professor of Mol. Biol. Robert Singleton received Ph.D. degree and joined the genetics division of Dow chemical as a staff scientist in Jan. 1993 where he is gainfully serving as a scientist. The results of this project supported by DOE contract was presented on invitation to Mol. Biol. of replication meetings in Cold Springs Harbor Laboratory, Cold Spring Harbor, NY; Leucern, Switzerland; Medical University, Debrecen, Hungary, University of Halle Wittenberg, Germany; International Genetics and Biotech. Center, Trieste, Italy; and at the International Genetics Congress in Birminham, U.K.

We expect to publish 2-3 more articles, based on our work supported by the current DOE grant.

Related publications: Supported by other grant

1. Yadav, J.S. and N.C. Mishra 1994
Eukaryotic cells and expression vectors 133-180 pages in Food Biotechnology (Ed. Hui and Khatchatourians)
VCH Publisher Inc. New York

Other related work

We have characterized the role of different DNA polymerases in yeast mutant cells. This work is carried by a graduate student, Marion Cooley in my laboratory. This work was supported to be presented at the yeast Molecular biology meetings in August 1994 in. Seattle WA.