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CHARACTERIZATION OF THE MAMMALIAN
DNA POLYMERASE GENE AND PROTEIN

Annual Progress Report

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

by

N.C. Mishra, Ph.D. Principal Investigator

Abstract

Consistent with the long term goal of our research to understand the nature of the key enzymes in eukaryotic DNA replication we have characterized the properties of the wild type DNA polymerases of the α -family and their mutants. We have also provided evidence for the role of aphidicolin in the elongation process of the *in vivo* DNA replication in eukaryotic cells. We also developed a technology for planned prep from a large numbers of clones for direct screening by size or restriction digestion in order to facilitate our goals to clone the DNA polymerase gene. These results were submitted for publication in referenced journals.

Introduction

We have created DNA polymerase mutants of the Chinese hamster ovary cells by their resistance to aphidicolin, a known inhibitor of the DNA polymerases of the α -family. These wild type enzymes and their mutant counter parts were characterized to understand their role in DNA replication in eukaryotic cells. The major findings are described below.

Results

1. Aphidicolin resistant Chinese hamster ovary cells possess altered DNA polymerases of the α -family.

DNA polymerases α , δ , and ϵ were partially purified and characterized from a wild type Chinese hamster ovary (CH) cell line and from two aphidicolin-resistant mutant CHO cell lines (BR5 and BR5-20a). The main characteristics of the wild type and mutant DNA polymerases were compared in order to reveal differences in the properties of these enzymes responsible for the aphidicolin resistance of the mutant cell lines. Pol α 's of the mutant cells show: (1) in vitro aphidicolin-resistance (2) 1.5-3-fold lower specific activity than that of the wild type, (3) resistance to cytosine and adenosine arabinofuranoside 5'-triphosphate (araCTP and araATP), (4) altered resistance to carbonyldiphosphonate (COMDP) and to alkylphenyl nucleotide analogs (butylphenyl-dGTP and butylanilino-dATP), and (5) lower activity on poly(dA)/oligo(dT) template-primers. These changes in the biochemical properties of this enzyme may result from a mutation in pol α gene. Pol ϵ and δ of the mutant cells did not differ from the wild type enzymes with respect to aphidicolin resistance. However, the specific activities of these mutant enzymes were much higher (1.5 to 8-fold for pol ϵ and 4 to 20-fold for pol δ) in comparison to that of the wild type enzymes. Also in comparison to the wild type enzymes, the mutant pol ϵ showed changes in the template-primer preference; whereas the mutant pol δ was found to have altered sensitivity to other inhibitors. These results indicate that pol ϵ and pol δ are also altered as a secondary effect of mutation in the aphidicolin-resistant cells. It is suggested that these altered properties of the DNA pols of the α family are responsible for the in vivo aphidicolin resistance of the mutant cells.

2. An aphidicolin-resistant mutant of Chinese hamster ovary cell with altered DNA-polymerase and 3' exonuclease activities.

A comparison was made of partially purified polymerases α , δ , ϵ and from normal Chinese hamster ovary cells and mutant cells (JK3-1-2A) resistant to aphidicolin, araA, and araC. In vitro the pol α from the mutant cells (1) was resistant to aphidicolin and araCTP but was sensitive to araATP, (2) showed a 1.6 to 2.6-fold reduced specific activity, and (3) was more sensitive to carbonyldiphosphonate. DMSO and SJK 287-38 anti-pol α antibody inhibition, but was less sensitive to alkylphenyl nucleotide analogs BuPdGTP and BuAdATP. On the other hand, pol δ and ϵ of the mutant cells did not show increased aphidicolin-resistance but differed from the wild type enzymes with regard to their 3' exonuclease activity. The 3' exonuclease/DNA polymerase activity ratio was increased 6-fold for pol δ and 3.3-fold for pol ϵ for enzymes from the mutant cells in comparison to

wild type values. It is suggested that these altered properties of the DNA polymerases of the α -family are responsible for in vivo aphidicolin resistance of the mutant cells. The higher 3' exonuclease activity may explain the observed antimutator phenotype of this cell line. In view of the proficient 3' exonuclease activities of the DNA pol δ and ϵ , the present *aph^r* mutant is unique among all mammalian DNA polymerase mutants.

3. Genetic evidence that aphidicolin inhibits in vivo DNA synthesis in Chinese hamster ovary cells.

Using a genetic approach, Chinese hamster ovary (CHO) cells sensitive (*aph^s*) and resistant (*aph^R*) to aphidicolin were grown in the presence or absence of various DNA polymerase inhibitors, and the newly synthesized DNA isolated from [³²P]dNMP-labelled, detergent-permeabilized cells, was characterized after fractionation by gel electrophoresis. The particular *aph^R* mutant CHO cell line used was one selected for resistance to aphidicolin and found to possess an altered DNA polymerase of the α -family. The synthesis of a 24Kb replication intermediate was inhibited in wild-type CHO cells grown in the presence of aphidicolin, whereas the synthesis of this replication intermediate was not inhibited by this drug in the mutant CHO cells or in the aphidicolin-resistant somatic cell hybrid progeny constructed by fusion of wild-type and mutant cell lines. Arabinofuranosylcytosine (ara-C), like aphidicolin, inhibited the synthesis of this 24 Kb DNA replication intermediate in the wild type CHO cells but not in the *aph^R* mutant cells. However, carbonyldiphosphonate (COMDP) inhibited the synthesis of the 24 Kb replication intermediate in both wild-type and mutant cells. N²-(*p*-n-Butylphenyl)-2' deoxyguanosine-5'-triphosphate (BuPdGTP) was found to inhibit the formation of Okazaki fragments equally well in the wild type and mutant cell lines and thus led to inhibition of synthesis of DNA intermediates in both cases. It appears that aphidicolin and ara-C both affect a common target on the DNA polymerase, which is different from that affected by COMDP in vivo. These data also show that aphidicolin, ara-C and COMDP affect the elongation activity of DNA polymerase but not the initiation activity of the enzyme during DNA replication. This is the first report of such differentiation of the DNA polymerase activities during nuclear DNA replication in mammalian cells. The method of analysis described here for replication intermediates can be used to examine the inhibitory activities of other chemicals on DNA synthesis.

4. Plasmid preparation from large number of clones.

We have developed a methodology to analyze a large number of clones harboring recombinant plasmid in order to facilitate the cloning of DNA polymerase gene(s).

The results were submitted for publication.

Publication

1. **Feher, Z. and N.C. Mishra 1994. Aphidicolin resistant Chinese hamster ovary cells possess altered DNA polymerase of the α family revised manuscript submitted to Biophys. Biochem Acta. (Initially two manuscripts were submitted but on Editor's suggestion both article were combined entirely except for a common Introduction and Discussion section.**
2. **Singleton, R. and N.C. Mishra 1994. Genetic evidence that aphidicolin inhibits the in vivo synthesis in Chinese hamster ovary cells. Molecular and General Genetics. (Submitted)**
3. **Liu, Zitong and N.C. Mishra 1994
Single tube method for plasmid mini prep from large numbers of clones for direct screening by size or restriction digestion.
Biotechnology. (Submitted)**
4. **Feher Z. and N.C. Mishra. 1994.
An aphidicolin-resistant mutant of chinese hamster ovary cell with altered DNA polymerase and 3' exonuclease activities.
Biochim Biophys Acta (Submitted)**